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L-3,4-Dihydroxyphenylalanine as a neurotransmitter candidate in the central nervous system

Yoshimi Misu[a,b,*], Kunio Kitahama[c], Yoshio Goshima[a]

[a]*Department of Pharmacology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan*
[b]*Shinobu Hospital, 31-1 Takahata, Ohmori, Fukushima 960-1101, Japan*
[c]*UMR 5123 CNRS, Laboratory of Physiology, Claude Bernard University, Lyon, France*

Abstract

Historically, 3,4-dihydroxyphenylalanine (DOPA) has been believed to be an inert amino acid that alleviates the symptoms of Parkinson's disease by its conversion to dopamine via the enzyme aromatic L-amino acid decarboxylase. In contrast to this generally accepted idea, we propose that DOPA itself is a neurotransmitter and/or neuromodulator, in addition to being a precursor of dopamine. Several criteria, such as synthesis, metabolism, active transport, existence, physiological release, competitive antagonism, and physiological or pharmacological responses, must be satisfied before a compound is accepted as a neurotransmitter. Recent evidence suggests that DOPA fulfills these criteria in its involvement mainly in baroreflex neurotransmission in the lower brainstem and in delayed neuronal death by transient ischemia in the striatum and the hippocampal CA1 region of rats.

Keywords: DOPA; Baroreflex neurotransmitter candidate; Glutamate release; Neuronal nitric oxide synthase; Nitric oxide production; Delayed neuronal death

Abbreviations: AADC, aromatic L-amino acid decarboxylase; ACh, acetylcholine; ADN, aortic depressor nerve; α-MPT, α-methyl-*p*-tyrosine; COMT, catechol-*O*-methyltransferase; CVLM, caudal ventrolateral medulla; DOPA, 3,4-dihydroxyphenylalanine; DOPA CHE, 3,4-dihydroxyphenylalanine cyclohexyl ester; DOPA ME, 3,4-dihydroxyphenylalanine methyl ester; GABA, γ-aminobutyric acid; L-*threo*-DOPS, L-*threo*-3,4-dihydroxyphenylserine; MAO, monoamine oxidase; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzol[*a*,*d*]cyclohepten-5,10-imine maleate; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NSD-1015, 3-hydroxybenzylhydrazine; NTS, nucleus tractus solitarii; PHN, posterior hypothalamic nucleus; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rats; TH, tyrosine hydroxylase; TTX, tetrodotoxin; WKY, Wistar Kyoto rats.

Contents

* Corresponding author. Shinobu Hospital, 31-1 Takahata, Ohmori, Fukushima 960-1101, Japan. Tel.: +81-24-546-3311; fax: +81-24-546-9467.
E-mail address: ymisu@zd5.so-net.ne.jp (Y. Misu).

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1. Introduction

The term DOPA is used in this article to refer to endogenous 3,4-dihydroxyphenylalanine, whereas levodopa refers to the exogenously applied substance. Since the times of Carlsson et al. (1957), levodopa has been believed traditionally to be an inert amino acid that alleviates the symptoms of Parkinson's disease via its conversion to dopamine by the enzyme aromatic L-amino acid decarboxylase (AADC) (Bartholini et al., 1967; Hefti & Melamed, 1980). This conversion initiates the resultant phenomena such as displacement of tritiated dopamine (Ng et al., 1970) and impulse-evoked release of radioactive dopamine (Ng et al., 1971) from brain slices. Both accumulation of striatal dopamine (Lloyd et al., 1975; Ponzio et al., 1983) and increases in the striatal tissue content of 3-methoxytyramine, a metabolite formed from dopamine in the synaptic cleft (Ponzio et al., 1983), also occurred. Dopamine converted from levodopa applied was thought to activate presynaptic inhibitory dopamine receptors and to lead to decreases in the firing rate of cells in the zona compacta and ventral tegmental areas (Bunney et al., 1973) and to the inhibition of cardiac acceleration by electrical sympathetic nerve stimulation (Lokhandwala & Buckley, 1978). These effects following the application of levodopa are abolished by an AADC inhibitor and are mimicked by dopamine (Lokhandwala & Buckley, 1978) and apomorphine, a dopamine agonist (Bunney et al., 1973).

In contrast, since 1986 (Goshima et al., 1986; Misu et al., 1986), we have proposed a working hypothesis that DOPA itself is a neurotransmitter and/or neuromodulator, in addition to its role as a precursor of dopamine. In the course of studies on presynaptic regulatory mechanisms of the evoked release of endogenous catecholamines from brain slices (Misu & Kubo, 1983, 1986; Ueda et al., 1983a, 1983b, 1984, 1985; Goshima et al., 1985; Misu et al., 1985), we found an unknown peak increased by electrical field stimulation on chromatographic charts. We identified it as DOPA and found that it was released like a neurotransmitter (Goshima et al., 1988).

The first evidence for responses to levodopa itself, but not dopamine converted from levodopa, comes from the biphasic effects of levodopa on the release of catecholamines evoked by electrical field stimulation from hypothalamic slices (Goshima et al., 1986). Nanomolar levodopa facilitates the release of noradrenaline via presynaptic β-adrenoceptors in a propranolol-sensitive manner under intact AADC activity, and this facilitation is also seen under inhibition of AADC. Nanomolar levodopa elicits similar facilitation of dopamine release under inhibition of AADC. In contrast, micromolar levodopa inhibits the release of noradrenaline and dopamine via presynaptic dopamine D_2 receptors in a sulpiride-sensitive manner under inhibition of AADC. Furthermore, non-effective picomolar levodopa potentiates the β-agonist-induced facilitation of noradrenaline release via activation of presynaptic β-adrenoceptors (Goshima et al., 1991c). These responses to levodopa are completely opposite to those elicited by dopamine agonists (Misu et al., 1985). Nanomolar dopamine elicits the sulpiride-sensitive inhibition of noradrenaline release via presy-

naptic dopamine D_2-receptors, whereas micromolar dopamine elicits the propranolol-sensitive facilitation of noradrenaline release via presynaptic β-adrenoceptors. Nanomolar apomorphine also elicits the sulpiride-sensitive inhibition of noradrenaline release similar to dopamine.

Thereafter, we have been accumulating further evidence to support the hypothesis that DOPA is by itself a neurotransmitter. In this review, we will outline how DOPA fits the classical criteria for neurotransmitters, including synthesis, metabolism, active transport, presence, physiological release, competitive antagonism, and physiological or pharmacological responses (Hoffman & Taylor, 2001), that must be satisfied for the hypothesis to be accepted. Related reviews for DOPA have appeared previously (Misu & Goshima, 1993; Misu et al., 1995a, 1995b, 1995c, 1996, 2002a, 2002b; Opacka-Juffry & Brooks, 1995; Sun, 1996; Tedroff, 1998).

2. Synthesis and metabolism

DOPA is synthesized from tyrosine by the action of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine biosynthesis, and is converted to dopamine by the action of AADC (Fig. 1) (Misu & Goshima, 1993; Misu et al., 1995a, 1996; Hoffman & Taylor, 2001). Catecholamines are metabolized by catechol-*O*-methyltransferase (COMT) and by monoamine oxidase (MAO).

On chromatographic charts, putative DOPA peaks in dialysates comigrate with those of authentic levodopa when the pH of the buffer solution is slightly changed (Goshima et al., 1988; Nakamura et al., 1992a). The retention time of putative DOPA differs from that of dopamine, noradrenaline, and the major metabolites. Purified AADC converts putative DOPA to dopamine (Nakamura et al., 1992a). The TH inhibitor α-methyl-*p*-tyrosine (α-MPT) decreases basal release (Nakamura et al., 1992a; Yue et al., 1993a, 1993b, 1994a, 1994b; Miyamae et al., 1999b) and impulse-evoked release (Goshima et al., 1988; Miyamae et al., 1999b) of putative DOPA in the striatum and sites of baroreflex neurotransmission in the lower brainstem of rats. In contrast, the central AADC inhibitor 3-hydroxybenzylhydrazine (NSD-1015) increases the basal release of putative DOPA (Nakamura et al., 1992a, 1994; Yue et al., 1994a; Furukawa et al., 2001). These findings indicate that the putative DOPA on chromatographic charts is indeed DOPA itself.

In an in vitro experimental system, NSD-1015 (20 μM) is estimated to inhibit AADC activity by 99.6% (Goshima et al., 1990). Responses to levodopa in the presence of such AADC inhibitors are (1) not inhibited by a COMT inhibitor

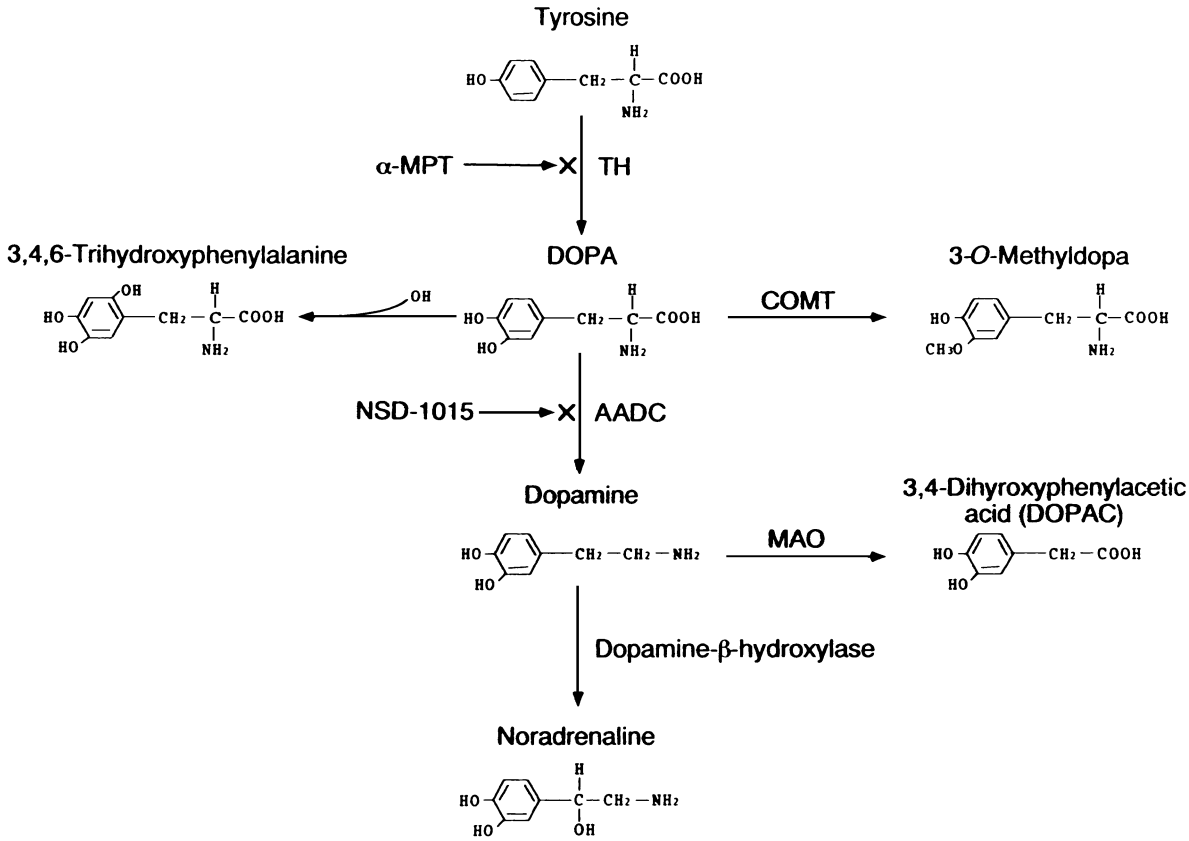

Fig. 1. Major pathways for the synthesis and metabolism of DOPA. DOPA is produced from tyrosine by the rate-limiting enzyme of catecholamine biosynthesis, TH. The action of TH is inhibited by α-MPT. DOPA is metabolized to 3-*O*-methyldopa by the action of COMT or is converted to dopamine by the action of AADC. NSD-1015 inhibits central AADC. Modified from Misu et al. (2002b).

(Goshima et al., 1990); (2) not mimicked by 3-*O*-methyldopa, a metabolite of DOPA following conversion by COMT; (3) not mimicked by 3,4-dihydroxyphenylacetic acid, a metabolite of dopamine produced by the action of MAO (Goshima et al., 1991b); and further, (4) not mimicked by dopamine (Kubo et al., 1992; Yue et al., 1993a, 1993b), noradrenaline (Yue et al., 1993a, 1993b), or adrenaline (Yue et al., 1993b) at the same low dose ranges as levodopa. These findings suggest that the responses to levodopa we obtained are mediated by levodopa itself, but not following its bioconversion, and support the hypothesis that DOPA itself is a neurotransmitter.

3. Active transport

The transport of levodopa across the neuronal plasma membrane is cocaine-insensitive, which is common for large neutral amino acids, and so, differs from the cocaine-sensitive uptake of catecholamines (Misu & Goshima, 1993; Misu et al., 2002b). Na^+ deprivation decreases by one-half the uptake of labeled levodopa into slices prepared from brain regions, including the hypothalamus, hippocampus, cerebral cortex, and cerebellum (Goshima et al., 1991b; Sugaya et al., 2001). This uptake is also decreased by large neutral amino acids, but not by dextrodopa, dopamine, and NSD-1015. Among the amino acid transport systems B, $B^{0,+}$, $b^{0,+}$, y^+L, and L (McGivan & Pastor-Anglada, 1994; Devés & Boyd, 1998), the uptake of tritiated levodopa might be partially mediated via a neutral and basic amino acid transporter, $B^{0,+}$ (McGivan & Pastor-Anglada, 1994), in the hippocampus (Sugaya et al., 2001). Meanwhile, the related $b^{0,+}$ amino acid transporter subunit might be involved in the Na^+-dependent uptake of labeled levodopa into *Xenopus laevis* oocytes injected with rabbit small intestinal epithelium poly A^+ RNA (Ishii et al., 2000). The existence of a Na^+-dependent active transport system for DOPA is essential to support the hypothesis that DOPA is a neurotransmitter, in addition to the criteria described in the following paragraphs.

4. Existence

DOPA exists as a precursor molecule in the cytoplasm of catecholaminergic neurons (Misu et al., 1995a, 1996; Hoffman & Taylor, 2001). However, there is immunocytochemical evidence for the existence of neurons that may contain DOPA as an end product. Jaeger et al. (1984) showed that a small group of TH-containing neurons in the rat dorsal motor nucleus of the vagus, which previously were classified as dopaminergic, lacked immunoreactivity to AADC. Jaeger and colleagues were first to suggest, from immunocytochemical data, the possibility that DOPA could be a neurotransmitter. However, we could not safely say that TH-containing neurons actually synthesize DOPA, because it became increasingly evident that TH-expressing neurons did not necessarily produce DOPA (Skagerberg et al., 1988; Tison et al., 1990; Kitahama et al, 1990, 1998a, 1998b; Nagatsu et al., 1999). On the other hand, Geffard and co-workers succeeded in producing antibodies to dopamine (Geffard et al., 1984) and DOPA (Mons & Geffard, 1987) suitable for immunocytochemical studies of DOPA and dopamine utilizing the glutaraldehyde condensation reaction. Thus, Misu et al. (1996) were able to immunocytochemically define TH-positive, DOPA-positive, AADC-negative, and dopamine-negative neurons (Fig. 1) as probable DOPAergic neurons. The first evidence for DOPAergic neurons was shown in the arcuate nucleus of the rat hypothalamus (Meister et al., 1988; Okamura et al., 1988a, 1988b). Thereafter, Tison et al. (1989) demonstrated DOPA immunoreactivity in the rat dorsal vagal complex, which includes the nucleus tractus solitarii (NTS), the gate of the baroreflex neurotransmission in the lower brainstem, and the dorsal motor nucleus of the vagus (Fig. 2). Bright, intense, and homogeneous DOPA immunostaining was found in the perikarya, and proximal neuronal processes were situated within the rostrocaudal extension of the dorsal vagal complex. This staining pattern was readily distinct from weak and heterogeneous dopamine immunostaining. TH-positive, AADC-negative (Jaeger et al., 1984), DOPA-positive, and dopamine-negative neurons (Tison et al., 1989) are also observed in the dorsal vagal complex of cats (K. Kitahama, unpublished data). Furthermore, the cells containing TH, but lacking AADC, in the most medial part of the dorsal vagal motor nucleus of mice show immunocytochemical reactivity of guanosine triphosphate cyclohydrolase I, the first and rate-limiting enzyme for the biosynthesis of tetrahydrobiopterin, a cofactor for the biosynthesis of DOPA (Nagatsu et al., 1995). The physiological (Yue et al., 1994b) and pathophysiological significance (Yue et al., 1995) of these neurons, however, had to wait for the certification of DOPA as a neurotransmitter candidate of the aortic depressor nerve (ADN), one of the primary baroreceptor afferents terminating in this area (Fig. 2).

Classically, in the peripheral nervous system, catecholaminergic phenotypic characteristics have been restricted to sympathetic neurons. However, the presence of the catecholaminergic trait in primary sensory neurons was also reported in the dorsal root ganglion (Price & Mudge, 1983) and in the ganglion nodosum (Yoshida et al., 1981; Katz et al., 1983; Helke & Niederer, 1990). In the latter ganglion, TH immunoreactivity was detected in the absence of dopamine-β-hydroxylase (the enzyme that synthesizes noradrenaline from dopamine) or phenylethanolamine-*N*-methyltransferese (the enzyme that synthesizes adrenaline from noradrenaline) immunoreactivity, leading to the conclusion that these neurons were neither noradrenergic nor adrenergic. In addition, in the rat ganglion nodosum, Yue et al. (1994b) found TH and DOPA immunoreactivity, and

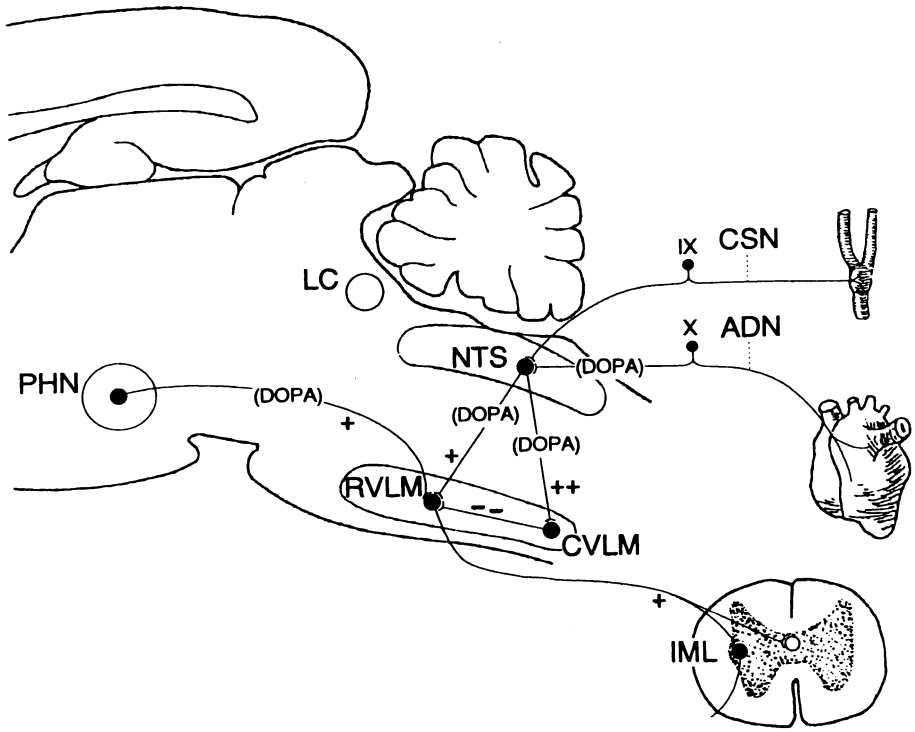

Fig. 2. Baroreflex pathways, central regulatory mechanisms of arterial blood pressure, and probable DOPA-mediated relays in the lower brainstem of anesthetized rats. Baroreflex is the principal neuronal mechanism by which the cardiovascular system is regulated under negative feedback control. Baroreceptors are located in the aortic arch and the carotid sinus. The primary baroreceptor afferents, the ADN and the carotid sinus nerve (CSN), terminate in depressor sites of the NTS. Glutamate has been regarded as the most probable neurotransmitter of the primary baroreceptor afferents, interneurons, or microcircuit-neurons within depressor sites of the NTS, among many candidates. The NTS neurons send excitatory projections to the CVLM and the RVLM. The neurotransmitter of these pathways is believed to be glutamate. The tonicity of the excitatory NTS-RVLM pathway, shown as '+,' appears to be less than the tonicity of the predominant excitatory NTS-CVLM pathway, shown as '++.' Vasomotor tone is reduced by the neuronal activity of the CVLM, the integration of which constitutes the central pathway for baroreflex neurotransmission. GABA-containing neurons in the CVLM project directly to the RVLM to inhibit excitatory activity. The tonicity of GABA-containing neurons is shown as '– – .' The RVLM receives excitatory inputs from various regions, including the NTS and the PHN. The excitatory neurons of the RVLM project directly to the intermediolateral cell column (IML) of the thoraco-lumbar spinal cord, the main origin of the sympathetic discharge. The RVLM plays an important role in controlling resting and reflex integration of arterial blood pressure. DOPA is probably a neurotransmitter of the primary baroreceptor afferents. A DOPA-mediated baroreceptor-ADN-NTS-CVLM depressor relay and a DOPA-mediated PHN-RVLM pressor relay appear to exist. LC, locus caeruleus. Modified from Misu et al. (2002b).

very rare dopamine immunoreactivity. Furthermore, following denervation of the ADN peripheral to the ganglion, decreases in TH and DOPA immunoreactivity in the host cell bodies in the ganglion nodosum itself and in the centrally projecting terminals in the dorsal vagal complex were detected without decreases in dopamine and dopamine-β-hydroxylase immunoreactivity.

Immunocytochemically TH-positive, DOPA-positive, AADC-negative, and dopamine-negative neurons are first found in the ventrolateral part of the arcuate nucleus of the rat hypothalamus and the periarcuate region just dorsal to the ventral surface of the brain (Meister et al., 1988; Okamura et al., 1988a, 1988b). Related to this tuberoinfundibular system, it is interesting to note that intensely stained DOPA-immunoreactive neuron terminals are observed in the external layer of the rat median eminence (Misu et al., 1996; Sugaya et al., 2001). DOPA-immunoreactive sites observed in both sides of the median eminence extend into its intermediate layer. This distribution pattern of DOPA-immunoreactive nerve terminals coincides well with that of moderate to heavy grain accumulation representing tritiated levodopa observed in the lateral part of the median eminence by microautoradiography (Sugaya et al., 2001). These findings suggest the possibility that DOPA might be involved in neuro-endocrine regulatory mechanisms including the anterior pituitary system.

In the rat and cat posterior and dorsal hypothalamus, DOPA-immunoreactive cells are generally fewer in number, compared with dopamine-immunoreactive cells (Skagerberg et al., 1988; Kitahama et al, 1990; Tison et al., 1990). In the rat posterior hypothalamic nucleus (PHN) (A11 cell group), however, some cells display TH-positive, weakly AADC-positive (Skagerberg et al., 1988), and markedly DOPA-positive immunoreactivity with very weakly dopamine-positive immunoreactivity (Tison et al., 1990). The rat PHN neurons send descending projections to the rostral ventrolateral medulla (RVLM) (Vertes & Crane, 1996), the exit of the pathways for baroreflex neurotransmission from the

lower brainstem to the thoraco-lumbar spinal cord (Fig. 2). On the basis of these findings, it becomes evident that electrical PHN stimulation releases DOPA in a tetrodotoxin (TTX)-sensitive manner during microdialysis of the RVLM without increases in dopamine, noradrenaline, or adrenaline (Nishihama et al., 1999).

In hypothalamic magnocellular neurons of the rat supraoptic nucleus and paraventricular nucleus, very few TH-immunoreactive neurons were detected under normal conditions. After the rats were salt loaded, however, TH immunoreactivity and TH mRNA were detected abundantly in these nuclei (Kiss & Mezey, 1986; Young et al., 1987) without induction of AADC (Okamura et al., 1990b). It is possible that hyperosmotic conditions induce the appearance of TH-positive, DOPA-positive, AADC-negative, and dopamine-negative neurons in these nuclei and the internal layer of the median eminence (Tanaka et al., 1991). In the rat preoptic and anterior hypothalamic areas, DOPA-positive cell bodies and fibers were observed, whereas no, or a few, weakly immunostained dopamine-positive neurons were seen (Mons et al., 1990). Related to the existence of these DOPA-positive, but dopamine-negative, neurons in the rat hypothalamus, including the arcuate nucleus and the PHN, as described above, levodopa can regulate by itself the evoked release of catecholamines from the hypothalamic slices (Goshima et al., 1986, 1990, 1991b, 1991c; Sato et al., 1993).

On the other hand, the distribution of TH-immunoreactive neurons are reported to be variable in the brains of several mammalian species (Kitahama et al., 1994, 1998a). For example, TH-positive, AADC-negative, and dopamine-negative cells in the cat supraoptic nucleus did not show any detectable DOPA immunoreactivity (Kitahama et al., 1988, 1990). DOPA-positive neurons observed in the rat preoptic and anterior hypothalamic areas (Mons et al., 1990) are not confirmed in the case of cats (Kitahama et al., 1990). In the paraventricular nucleus of monkey (*Macaca fuscata*), TH-positive, AADC-negative, and dopamine-negative neurons are observed, whereas no evidence of DOPA-positive neurons was shown (Kitahama et al., 1998a). In the human supraoptic and paraventricular nuclei, TH-positive and AADC-negative neurons are observed, but no evidence of DOPA-positive neurons was shown (Kitahama et al., 1998a, 1998b). This is consistent with the observation that these nuclei in the human anterior hypothalamus do not show immunocytochemical reactivity to guanosine triphosphate cyclohydrolase I (Nagatsu et al., 1999).

Immunocytochemically, TH-positive, DOPA-positive, AADC-negative, and dopamine-negative neurons are detected in the lateral habenular nucleus of the thalamus in the house-shrew (*Suncus murinus*) brain, and DOPA-positive reaction products are observed by electron microscopy in the axoplasm and are associated with the outer surface of small, clear or cored vesicles (Karasawa et al., 1992). These criteria for DOPAergic neurons are satisfied in the ventral tegmental area of the human midbrain (Komori et al., 1993; Nagatsu et al., 1999). However, the physiological significance of these neurons is not clear at present.

Strong DOPA immunoreactive neurons are detected in the rat periaqueductal gray of the central canal (Okamura et al., 1990a). This is consistent with the observation that small TH-immunoreactive and guanosine triphosphate cyclohydrolase I-immunoreactive cell bodies are dispersed in the central gray along the floor of the human aqueduct (Nagatsu et al., 1999). The physiological role of these neurons is unclear.

It is important that DOPA is physiologically released in the caudal ventrolateral medulla (CVLM) (Miyamae et al., 1999b), the integration of which constitutes the central pathway for a negative feedback control of arterial blood pressure through baroreflex neurotransmission (Fig. 2). The physiological release of DOPA is also evident in the RVLM (Nishihama et al., 1999), the striatum (Goshima et al., 1988; Nakamura et al., 1992a; Furukawa et al., 2001), the nucleus accumbens (Goshima et al., 1996a), and the hippocampal CA1 region (Arai et al., 2001). In these regions, however, immunocytochemical evidence for DOPAergic neurons (Misu et al., 1996) has not been found. Thus, sensitivity of immunocytochemical analysis used to find DOPAergic neurons is lower, compared with the biochemical approaches used (Misu et al., 1996, 2002b).

5. Physiological release

In rat superfused striatal slices, electrical field stimulation releases DOPA in a TTX-sensitive, Ca^{2+}-dependent, and α-MPT-sensitive manner (Goshima et al., 1988). Depolarizing stimuli, such as high K^+ (Goshima et al., 1988) and nicotine (Misu et al., 1990), also release DOPA Ca^{2+}-dependently. Levodopa appears to be taken up by neurons and to be released from rat striatal slices by high K^+ in a neurotransmitter-like manner (Chang & Webster, 1995). The Ca^{2+}-dependent evoked release of DOPA is also shown in peripheral tissues such as dog sympathetic ganglia (Kristensen et al., 1990), portal vein (Hunter et al., 1992), and adrenal medulla (Chritton et al., 1997).

Ca^{2+} plays essential roles in both the release of neurotransmitters (Douglas & Rubin, 1963; Kirpekar & Misu, 1967) and the activation of TH (El Mestikawy et al., 1983). Ca^{2+}, however, appears to be primarily involved in the release of DOPA. Indeed, the high K^+-evoked release of DOPA occurs temporally prior to the enhancement of TH activity in experiments to measure, simultaneously, tritiated H_2O formation from tritiated tyrosine superfused and the release of both DOPA and dopamine in striatal slices (Goshima et al., 1988). High K^+ (15 and 60 mM) releases DOPA at the same time as concentration-dependent decreases in TH activity are observed during depolarization. The decreases in TH activity are probably due to a negative feedback control via the activation of presynaptic dopamine

D_2 autoreceptors (Roth, 1984) following the concentration-dependent evoked release of dopamine. In contrast, concentration-dependent increases in TH activity are observed after the release of both DOPA and dopamine has ended. The depolarization induced by 60-mM K^+ releases less DOPA compared with 15-mM K^+, which appears to occur following the negative feedback control elicited by the concentration-dependent evoked release of dopamine.

The DOPA levels in striatal tissue are lower than those of dopamine, an established neurotransmitter, by 3 orders of magnitude (Westerink et al., 1982), whereas the basal release of DOPA:dopamine is 1:2−3 in striatal slices (Goshima et al., 1988; Misu et al., 1990). This means that the turnover rate of DOPA is higher than that of dopamine. Indeed, the percent of DOPA evoked as a fraction of the tissue contents after the end of the experiments is higher by 2−3 orders of magnitude than those of dopamine. DOPA appears to be more efficiently synthesized and released to elicit function compared with dopamine (Misu et al., 1995a, 1996). The evoked release of DOPA from rat striatal slices appears to be derived from some cytoplasmic compartment other than dopamine-containing vesicles (Goshima et al., 1988; Misu & Goshima, 1993). Furthermore, it is confirmed that during striatal microdialysis of conscious rats, the ratio of the basal release of DOPA:dopamine is 1:2 (Nakamura et al., 1992a, 1992b, 1994; Yue et al., 1994a).

Microdialysis was also performed in the NTS (Yue et al., 1994b, 1995), the CVLM (Yue et al., 1993a; Miyamae et al., 1995, 1999b), the RVLM (Yue et al., 1993b, 1995; Nishihama et al., 1999), and the nucleus accumbens (Goshima et al., 1996a). The basal release of DOPA is partially TTX-sensitive, in part Ca^{2+}-dependent (Nakamura et al., 1992a; Yue et al., 1993a, 1993b, 1994a, 1994b, 1995; Miyamae et al., 1995; Goshima et al., 1996a), and markedly α-MPT-sensitive (Nakamura et al., 1992a; Yue et al., 1993a, 1993b, 1994a, 1994b; Miyamae et al., 1999b). On the other hand, the basal release of dopamine shows essentially complete TTX-sensitivity, Ca^{2+}-dependency, and α-MPT-sensitivity. These findings suggest that there are two components of extracellular DOPA levels. One is the TTX-sensitive and Ca^{2+}-dependent release of DOPA, as a neurotransmitter, and the other is the TTX-insensitive and Ca^{2+}-independent efflux of DOPA, as the precursor of dopamine. Both high K^+ (Nakamura et al., 1992a; Yue et al., 1993a, 1993b, 1994b) and nicotine (Nakamura et al., 1992b) evoke Ca^{2+}-dependent DOPA release during microdialysis, which is supported by other findings (Okada et al., 1998).

The NTS, CVLM, and RVLM play essential roles in baroreflex neurotransmission and the central regulation of arterial blood pressure (Talman et al., 1980; Urbanski & Sapru, 1988; Sun, 1996; Vertes & Crane, 1996; Aicher et al., 2000; Andresen et al., 2001) (Fig. 2). DOPA is a highly probable neurotransmitter of the primary baroreceptor afferents (Yue et al., 1994b). Electrical ADN stimulation releases DOPA ipsilaterally in the NTS (Yue et al., 1994b) and the CVLM (Miyamae et al., 1999b) and is accompanied by hypotension and bradycardia. The evoked release of DOPA is abolished following the blockade of Na^+ channels by TTX infused in the NTS and following the inhibition of the biosynthesis of DOPA by α-MPT in the CVLM. DOPA may be released from immunocytochemically DOPAergic neurons (Misu et al., 1996) in the NTS (Yue et al., 1994b). No catecholamines are released by ADN stimulation in the CVLM (Miyamae et al., 1999b). Electrical PHN (Vertes & Crane, 1996) stimulation releases TTX-sensitive DOPA in the ipsilateral RVLM with hypertension and tachycardia (Nishihama et al., 1999). No catecholamines are released by PHN stimulation.

Being consistent with the physiological release of DOPA, it is an important finding that the denervation of the ADN decreases quantitatively TH and DOPA immunoreactivity, but not dopamine and dopamine-β-hydroxylase immunoreactivity, in the ipsilateral depressor sites of the NTS (Yue et al., 1994b). The ADN may utilize DOPA, but not dopamine and noradrenaline, as a neurotransmitter and possesses some DOPAergic monosynaptic properties. The DOPAergic long axons appear to project directly to the depressor sites and/or indirectly to some secondary neurons within neuronal microcircuits of the depressor sites in the NTS. In addition, the electrolytic lesion of the NTS selectively decreases by 50% the tissue contents of DOPA in the ipsilateral CVLM without decreasing those of catecholamines (Miyamae et al., 1999b). However, this lesion only slightly decreases the tissue contents of DOPA in the RVLM. These findings suggest less tonicity of the NTS-RVLM pathway compared with the NTS-CVLM pathway (Urbanski & Sapru, 1988). Furthermore, the electrolytic lesion of the bilateral PHN selectively decreases by one-half the tissue content of DOPA in the unilateral RVLM without decreasing catecholamines (Nishihama et al., 1999). These findings suggest the existence of a DOPAergic monosynaptic property in the depressor NTS-CVLM and pressor PHN-RVLM pathways. It is interesting to note that in newly established cell lines derived from the *Drosophila* larval CNS, DOPA is expressed in all 10 cloned cell lines as a novel neurotransmitter candidate (Ui-Tei et al., 1994). In contrast, acetylcholine (ACh), an established neurotransmitter, is found in only 7 of the 10 cloned lines. In addition, neither catecholamines nor their metabolites, serotonin, and octopamine were detected in any of these cell lines.

6. Competitive antagonism

DOPA methyl ester (DOPA ME) was the first competitive antagonist for levodopa to be identified (Goshima et al., 1991b, 1993). Among DOPA esters, DOPA cyclohexyl ester (DOPA CHE) is the most potent and relatively stable competitive antagonist for levodopa (Misu et al., 1997; Furukawa et al., 2000). These two antagonists antagonize responses to both levodopa (Goshima et al., 1991c; Kubo et

al., 1992; Yue et al., 1993a, 1993b; Akbar et al., 2001) and DOPA (Yue et al., 1993a, 1993b, 1994b; Miyamae et al., 1999b; Nishihama et al., 1999; Furukawa et al., 2001; Arai et al., 2001). Competitive antagonism suggests the existence of DOPA recognition sites.

DOPA CHE does not inhibit the uptake of labeled levodopa into oocytes (Ishii et al., 2000). DOPA esters and levodopa do not displace the selective binding of tritiated dopamine D_1 and D_2 receptor ligands or tritiated α_2-adrenoceptor and β-adrenoceptor ligands in rat brain membrane preparations (Goshima et al., 1991b; Furukawa et al., 2000). In addition, DOPA ME fails to displace the selective binding of tritiated γ-aminobutyric acid (GABA) (Honjo et al., 1999). Furthermore, DOPA ME antagonizes depressor and bradycardic responses to levodopa microinjected into depressor sites of the NTS (Kubo et al., 1992) and the CVLM (Yue et al., 1993a) and pressor and tachycardic responses to levodopa microinjected into pressor sites of the RVLM (Yue et al., 1993b). In contrast, DOPA ME does not antagonize glutamate-induced responses similar to levodopa in the NTS (Kubo et al., 1992), CVLM (Yue et al., 1993a), and RVLM (Yue et al., 1993b). Among the binding sites labeled with tritiated ionotropic glutamate receptor ligands, DOPA esters act only on the ion channel of *N*-methyl-D-aspartate (NMDA) receptors with an IC_{50} in the millimolar range, whereas levodopa acts only on DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors with low affinity (Miyamae et al., 1999a; Furukawa et al., 2000). DOPAergic agonist and competitive antagonists should act at the same sites, which appear to differ from DOPA transport sites, catecholamine receptors, GABA receptors, and ionotropic glutamate receptors. To support the identification of DOPA as a neurotransmitter, it is essential that DOPA recognition sites exist on which DOPA or levodopa act to elicit physiological or pharmacological responses.

7. Responses

7.1. Preface

Most responses to levodopa, like many neurotransmitters, are stereoselective (Goshima et al., 1991b, 1991c, 1993; Kubo et al., 1992; Yue et al., 1993a, 1993b; Nakamura et al., 1994; Ueda et al., 1995; Cheng et al., 1996; Furukawa et al., 2001). Levodopa elicits responses in the presence of central AADC inhibition (Misu et al., 1986; Goshima et al., 1986, 1990, 1991b, 1991c, 1993; Kubo et al., 1992; Yue et al., 1993a, 1993b; Nakamura et al., 1994; Akbar et al., 2001). These pharmacological responses are elicited by levodopa itself, but not by bioconversion to dopamine. Furthermore, responses to DOPA are potentiated following an increase in the basal release of DOPA elicited by NSD-1015 (Yue et al., 1994a; Furukawa et al., 2001). In contrast, responses to DOPA are inhibited following a decrease in the biosynthesis of DOPA induced by α-MPT (Yue et al., 1993a, 1993b, 1994a, 1994b; Miyamae et al., 1999b) or following the blockade of Na^+ channels elicited by TTX (Nishihama et al., 1999). These findings suggest that physiological functions are induced following basally released and impulse-evoked DOPA.

7.2. The lower brainstem

7.2.1. Responses to levodopa and 3,4-dihydroxyphenylalanine

Baroreceptors are located at the aortic arch and the carotid sinus (Fig. 2). The primary baroreceptor afferents terminate in depressor sites of the NTS (Talman et al., 1980; Aicher et al., 2000; Andresen et al., 2001). The NTS neurons send excitatory projections to the CVLM and the RVLM (Urbanski & Sapru, 1988; Sun, 1996; Aicher et al., 2000; Andresen et al., 2001).

Fig. 3A–D shows representative traces of responses to levodopa in the NTS (Misu et al., 1996; Furukawa et al., 2000). Levodopa (10–100 ng) microinjected into depressor sites of the NTS (Fig. 3) elicits hypotension and bradycardia in the absence and the presence of NSD-1015 (Kubo et al., 1992; Yue et al., 1995; Misu et al., 1997; Furukawa et al., 2000). The same dose ranges of levodopa elicit similar responses in the CVLM (Yue et al., 1993a; Miyamae et al., 1995, 1999b). Levodopa (30–600 ng) applied in pressor sites of the RVLM elicits hypertension and tachycardia in the absence and the presence of NSD-1015 (Yue et al., 1993b, 1995). These responses are dose-dependent, stereoselective, not mimicked by catecholamines at the same low-dose ranges as levodopa, DOPA ME-sensitive (Fig. 3A–D in the NTS), and postsynaptic in nature. The difference in these doses used between the depressor NTS or CVLM and the pressor RVLM suggests less tonicity of the NTS-RVLM pathway compared with the NTS-CVLM pathway (Urbanski & Sapru, 1988).

Furthermore, the tonic function of DOPA recognition sites is proven by the antagonism of basally released DOPA. The bilateral microinjection of DOPA ME alone elicits hypertension and tachycardia in the depressor NTS (Fig. 3E) (Yue et al., 1994b) and the depressor CVLM (Yue et al., 1993a), and hypotension and bradycardia in the pressor RVLM (Yue et al., 1993b). All of these responses to the competitive antagonist alone, which suggest the physiological activation of depressor and pressor sites elicited by basally released DOPA, are abolished following inhibition of the biosynthesis of DOPA induced by the prior intraperitoneal injection of α-MPT (200 mg/kg) (Fig. 3F in the NTS).

7.2.2. Further evidence in the nucleus tractus solitarii

It is highly probable that DOPA is a neurotransmitter of the primary baroreceptor afferents (Yue et al., 1994b). Importantly, the microinjection of DOPA ME antagonizes the hypotension and bradycardia elicited ipsilaterally by

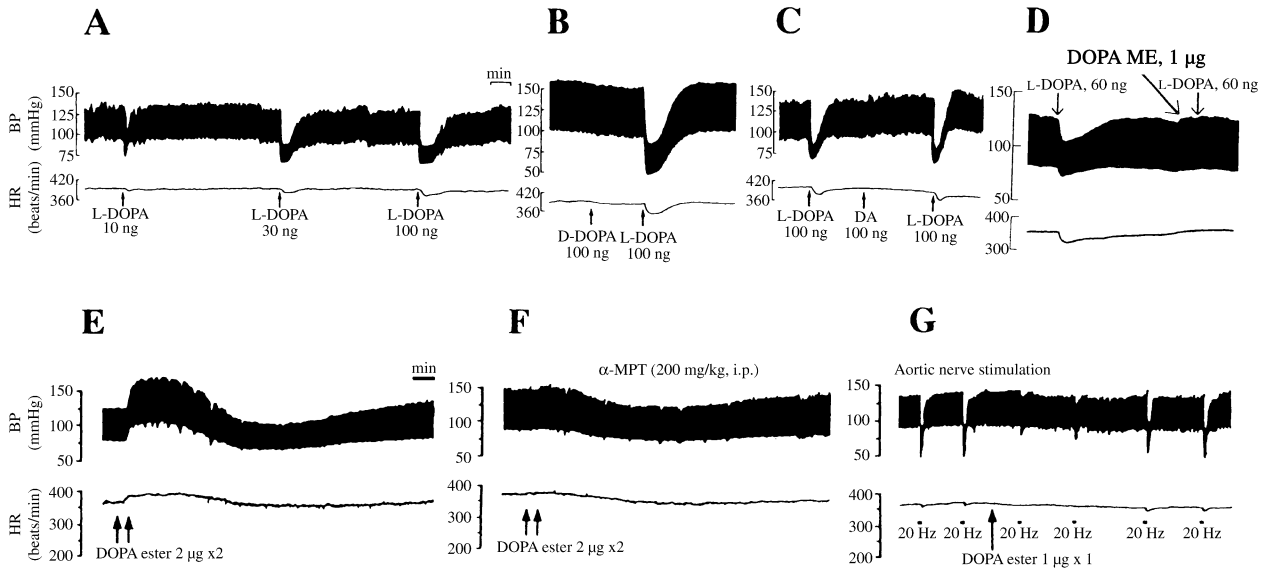

Fig. 3. Typical traces of responses to levodopa (L-DOPA) and DOPA ME (DOPA ester), a competitive antagonist of levodopa and endogenously released DOPA, microinjected into depressor sites of the NTS, and to electrical ADN (aortic nerve) stimulation in anesthetized rats. Hypotension and bradycardia elicited by the unilateral microinjection of levodopa are dose-dependent (**A**), stereoselective (**B**), not mimicked by dopamine (DA) at the same low-dose ranges to levodopa (**C**), antagonized by the ipsilateral microinjection of DOPA ME (**D**), and also seen under inhibition of aromatic L-amino acid decarboxylase. The bilateral microinjection of DOPA ME alone elicits hypertension and tachycardia (**E**), which are abolished by the prior intraperitoneal injection (200 mg/kg) of α-MPT, an inhibitor of the enzyme TH, involved in the synthesis of DOPA from tyrosine (**F**). These findings suggest that basally released DOPA, acting on its recognition sites, functions tonically to activate depressor sites of the NTS. A further important finding is that the unilateral microinjection of DOPA ME antagonizes hypotension and bradycardia elicited by either the ipsilateral electrical ADN stimulation (**G**) or exogenously applied levodopa (**D**) in a similar manner. BP, blood pressure; HR, heart rate. Modified from Misu et al. (1996) and Furukawa et al. (2000).

either the release of DOPA following the electrical stimulation of ADN or the microinjection of levodopa in similar fashion (Fig. 3D and 3G) (Kubo et al., 1992; Yue et al., 1994b; Furukawa et al., 2000). Furthermore, baroreceptor activation by intravenous infusion of phenylephrine elicits DOPA release during microdialysis and baroreflex bradycardia, which are both abolished by bilateral sino-aortic denervation without modification of hypertension (Yue et al., 1994b). The baroreflex bradycardia is also antagonized by the bilateral microinjection of DOPA ME.

7.2.3. Interactions between endogenously released γ-aminobutyric acid and 3,4-dihydroxyphenylalanine in the nucleus tractus solitarii

It has been thought that GABA is an inhibitory neuromodulator for baroreflex inputs in the depressor sites (Catelli et al., 1987; Kubo & Kihara, 1987, 1988). GABA (3–300 ng) microinjected into the unilateral depressor sites elicits dose-dependent hypertension and tachycardia (Honjo et al., 1999). Depressor and bradycardic responses to electrical ADN stimulation are attenuated by muscimol, a $GABA_A$ agonist, and nipecotic acid, an inhibitor of GABA uptake, but are potentiated by bicuculline, a $GABA_A$ antagonist, microinjected into the ipsilateral depressor sites (Kubo & Kihara, 1988). Thus, if it is the case that DOPA is a neurotransmitter of the ADN, depressor and bradycardic responses to levodopa should be inhibited by pretreatment with GABAergic agonists and potentiated by bicuculline. Indeed, GABA and nipecotic acid inhibited depressor and bradycardic responses to levodopa when pressor and tachycardic responses to GABA agonists alone returned to basal levels, whereas bicuculline potentiated these responses to levodopa when depressor responses to the antagonist alone returned to basal levels (Honjo et al., 1999). These findings suggest that the tonic function of endogenously released GABA, to inhibit depressor and bradycardic responses elicited by levodopa, occurs via the activation of postsynaptic $GABA_A$ receptors.

In addition, pressor and tachycardic responses to the highest dose of GABA (300 ng) decreased by one-half when the tonic function of basally released DOPA appeared to be antagonized by pretreatment with DOPA ME. This finding suggests that the microinjection of GABA elicits hypertension and tachycardia, at least partially, following inhibition of the tonic function of basally released DOPA, leading to the activation of postsynaptic depressor sites in the NTS.

Furthermore, GABA appears to tonically regulate the basal release of DOPA via activation of presynaptic inhibitory $GABA_A$ receptors probably located on DOPAergic neurons (Goshima et al., 1999). Indeed, during microdialysis of the NTS, locally perfused muscimol dose-dependently inhibits the basal release of DOPA, and this inhibition

is antagonized by a non-effective dose of bicuculline (10 μM). A higher dose of the antagonist (30 μM) alone inversely increases the basal release of DOPA.

These presynaptic and postsynaptic interactions between tonic functions of endogenously released GABA and DOPA in the NTS further support the hypothesis that DOPA is a neurotransmitter of the ADN.

7.2.4. Further evidence in the caudal ventrolateral medulla

It is highly probable that there exists a DOPAergic baroreceptor-ADN-NTS-CVLM depressor relay to carry baroreflex information (Miyamae et al., 1999b). During microdialysis of the unilateral CVLM, baroreceptor activation by the intravenous infusion of phenylephrine selectively releases DOPA without increasing extracellular levels of catecholamines. This release is abolished by electrolytically lesioning the ipsilateral NTS. Furthermore, the ipsilateral electrical ADN stimulation elicits selective DOPA release without increases in catecholamines, hypotension, and bradycardia. Local inhibition of the biosynthesis of DOPA with α-MPT infused into the depressor sites abolishes DOPA release and reduces hypotension, but not bradycardia, evoked by ADN stimulation. The microinjection of DOPA ME antagonizes the hypotension elicited by either exogenously applied levodopa (Yue et al., 1993a) or DOPA following ADN stimulation (Miyamae et al., 1999b). DOPA ME antagonizes bradycardia induced by microinjected levodopa, but it hardly antagonizes the bradycardia evoked by ADN stimulation in a manner similar to α-MPT. It seems likely that the main pathway of the preganglionic efferents of the baro-vagal reflex does not pass through the depressor sites of the CVLM (Miyamae et al., 1999b). DOPA ME-sensitive bradycardic responses to applied levodopa appear to be mainly due to a decrease in the tonicity of the cardiac sympathetic nerve. This type of decrease in response to ADN stimulation appears to be a minor component.

7.2.5. Further evidence in the rostral ventrolateral medulla

It is highly probable that there exists a DOPAergic pressor relay from the PHN to the RVLM. During microdialysis of the unilateral RVLM, the electrical stimulation of the ipsilateral PHN elicits selective DOPA release without increasing catecholamines, hypertension, and tachycardia (Nishihama et al., 1999). The ipsilateral infusion of TTX into the pressor sites markedly reduces DOPA release, partially inhibits hypertension, and slightly inhibits tachycardia evoked by PHN stimulation. This partial inhibition appears to be explained mainly by the neuronal activity of the pathway through the contralateral RVLM. Indeed, the bilateral microinjection of DOPA ME dose-dependently antagonizes hypertension and tachycardia induced by the unilateral electrical stimulation of the PHN. This is consistent with the antagonism elicited by the unilateral microinjection of DOPA ME against pressor and tachycardic responses to the ipsilateral application of levodopa (Yue et al., 1993b).

7.2.6. Altered tonic 3,4-dihydroxyphenylalaninergic systems in spontaneously hypertensive rats

Spontaneously hypertensive rats (SHR) have been used frequently as a genetic model for essential hypertension. The absolute value of the basal release of DOPA during microdialysis is lower in the depressor NTS (Yue et al., 1995) and the depressor CVLM (Miyamae et al., 1995), and is higher in the pressor RVLM (Yue et al., 1995) of adult SHR compared with age-matched Wistar Kyoto rats (WKY). Locally perfused TTX reduces the basal release of DOPA to the same level in these three regions of the two strains. TTX-sensitive tonic neuronal activity to release basal DOPA is lower in the depressor NTS, is lost in the depressor CVLM, and is higher in the pressor RVLM of SHR compared with WKY. In addition, the sensitivity of pressor responses to levodopa microinjected at the lower dose range is higher and the activity of AADC is lower in the pressor RVLM of SHR compared with WKY. All of these alterations may be involved in the maintenance of hypertension in adult SHR (Misu et al., 1995b, 1995c).

7.2.7. Is 3,4-dihydroxyphenylalanine a neurotransmitter of the primary aortic depressor nerve and is glutamate a neurotransmitter of the secondary neurons in the nucleus tractus solitarii?

Glutamate has been regarded as the most probable neurotransmitter candidate of the primary baroreceptor afferents (Talman et al., 1980; Aicher et al., 2000; Andresen et al., 2001). Kynurenate, a broad-spectrum antagonist for ionotropic glutamate receptors, however, abolishes depressor and bradycardic responses to electrical ADN stimulation, but hardly inhibits those that result from the microinjection of glutamate in many reports (Misu et al., 1996). It has been suggested that an as yet unknown excitatory amino acid, the responses to which are antagonized by kynurenate, should be a primary neurotransmitter of the primary baroreceptor afferents (Leone & Gordon, 1989). Prior microinjection of kynurenate markedly reduces depressor and bradycardic responses to levodopa (Yamanashi et al., 2002), suggesting the existence of a major pathway mediated by ionotropic glutamate receptors. Herein, we propose a novel pathway in which DOPA is a neurotransmitter of the primary ADN and glutamate is a neurotransmitter of secondary neurons or interneurons in neuronal microcircuits of the NTS (Fig. 4). Additional possibilities are that (1) DOPA recognition sites are located on or near the terminals of glutamate neurons as the primary ADN, (2) responses to levodopa that remain after application of kynurenate are antagonized by glutamate receptor antagonists acting on metabotropic glutamate receptors that might be located at perisynaptic and/or extrasynaptic postsynaptic membranes (Foley et al., 1999), and (3) DOPA released from the primary ADN activates the depressor sites directly. DOPA recognition sites appear to differ from kynurenate-sensitive ionotropic glutamate receptors because DOPA esters do not displace the kynurenate-sensitive selective binding of triti-

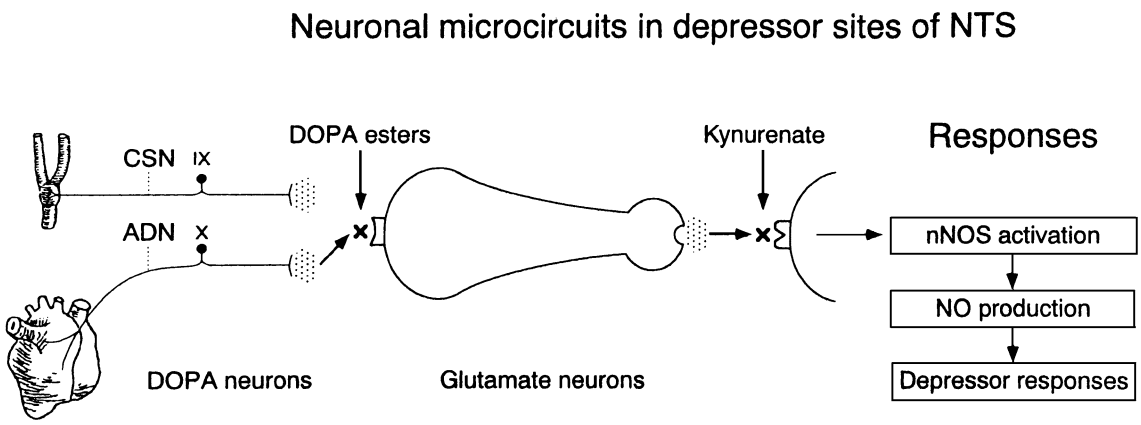

Fig. 4. A proposed novel pathway for DOPA-mediated neurons as the primary ADN and secondary glutamate neurons within neuronal microcircuits in depressor sites of the NTS of anesthetized rats. DOPA, by acting on its recognition sites in the NTS, might release undetectable, but functioning, glutamate from secondary neurons. DOPA esters antagonize depressor and bradycardic responses to either electrical ADN stimulation or the microinjection of levodopa, but not those to the microinjection of glutamate. Kynurenate elicits similar actions to DOPA esters. DOPA recognition sites appear to differ from kynurenate-sensitive ionotropic glutamate receptors. CSN, carotid sinus nerve. Modified from Misu et al. (2002b).

ated ligands for either the glycine site of the NMDA receptors, the NMDA binding site, or the kainate receptor (Miyamae et al., 1999a; Furukawa et al., 2000).

The end result of this pathway appears to be the production of nitric oxide (NO), which is a neuromodulator in the NTS (Maeda et al., 1999). Indeed, responses to levodopa are reversibly reduced both markedly and stereoselectively by N^G-monomethyl-L-arginine monoacetate, a neuronal NO synthase (nNOS) inhibitor, and markedly reduced by nNOS antisense oligonucleotides (Yamanashi et al., 2002). Sense and scrambled oligonucleotides elicit no effect. Kynurenate fails to modify depressor responses to the NO precursor L-arginine. These findings suggest that DOPA is a neurotransmitter of the primary ADN. Levodopa, by acting on DOPA recognition sites, might release undetectable, but functioning, glutamate from secondary neurons, which leads to the production of NO via kynurenate-sensitive postsynaptic ionotropic glutamate receptors and the subsequent depressor responses. Endogenous basal glutamate is measurable during microdialysis. However, the evoked release of glutamate above the basal level is not apparently observed following ADN and baroreceptor stimulation under the same experimental conditions that result in the release of DOPA (Yue et al., 1994b; Misu et al., 1994, 1996), which is consistent with other findings (Sved & Curtis, 1993). Based on the assumption that glutamate is a second-order neurotransmitter, the undetectable evoked release of glutamate might be reasonable.

7.3. Hypothalamic slices

7.3.1. Preface

In rat hypothalamic slices, we found the first competitive antagonist of levodopa, DOPA ME (Goshima et al., 1991b), by studying the facilitation of noradrenaline release evoked by electrical field stimulation via activation of presynaptic β-adrenoceptors (Misu & Kubo, 1983, 1986; Ueda et al., 1983a). Nanomolar levodopa (1–1000 nM) facilitates noradrenaline release in a concentration-dependent manner in the presence of NSD-1015 and sulpiride. DOPA ME antagonizes the levodopa-induced facilitation in a competitive fashion, whereas propranolol antagonizes it in a noncompetitive fashion, which suggests that DOPA recognition sites differ from β-adrenoceptors. This is consistent with the finding that DOPA ME elicits no displacement of the selective binding of a tritiated β-adrenoceptor ligand in rat brain membrane preparations. Furthermore, we studied structure-activity relationships for levodopa analogues by utilizing this measure.

7.3.2. Structure-activity relationships for levodopa

Comparative activities of levodopa analogues were determined by using the facilitation of the evoked release of noradrenaline via presynaptic β-adrenoceptors in hypothalamic slices (Goshima et al., 1991b) under essentially complete inhibition of central AADC (Goshima et al., 1990). NSD-1015 (20 μM) is expected to inhibit the activity of AADC by 99.6% in a noncompetitive manner. The levodopa-induced facilitation of noradrenaline release is not mimicked by phenylalanine, which lacks the hydroxy groups at the carbon 3 and 4 positions of the aromatic ring of levodopa (Fig. 1). This facilitation is not mimicked by 3-*O*-methyldopa, in which the hydroxy group at the carbon 3 of levodopa is substituted with a methoxy group. It is also not mimicked by DOPA phosphate, in which the hydroxyl group(s) at the carbon 3, 4, or both of levodopa is (are) substituted with the phosphorylated ester(s). 3,4-Dihydroxyphenylacetic acid, which lacks the amino group of the side chain of levodopa, and carbidopa, in which the amino group of methyldopa is substituted with a hydrazino group, do not

elicit a levodopa-like facilitation. In contrast, DOPA ME, a carboxylic acid methyl ester, is a competitive antagonist of levodopa and DOPA. Thus, the agonistic activity of levodopa appears to be related to such structural features as its catechol moiety, in addition to the amino and carboxyl groups. This idea is supported by the finding that L-*threo*-3,4-dihydroxyphenylserine (L-*threo*-DOPS), which has these three constituents and a hydroxyl group at the β position of levodopa, elicits an agonistic activity similar to levodopa.

L-*threo*-DOPS, a synthetic amino acid precursor of noradrenaline, has been thought to alleviate the symptoms of Parkinson's disease by its conversion to noradrenaline via the action of AADC (Bartholini et al., 1975; Narabayashi et al., 1981). However, L-*threo*-DOPS, at picomolar concentrations far lower than those to elicit conversion to noradrenaline, facilitates the evoked release of noradrenaline in the absence and presence of the AADC inhibitor in hypothalamic slices (Yue et al., 1992). This facilitation is competitively DOPA ME-sensitive and noncompetitively propranolol-sensitive in a manner similar to levodopa (Goshima et al., 1991b). These findings suggest that this artificial amino acid is the agonist acting by itself on DOPA recognition sites, in addition to its role as a precursor of noradrenaline. Competitive antagonism produced by DOPA ME also suggests the existence of an L-*threo*-DOPS-like endogenous substance in the CNS.

Furthermore, in the lower brainstem, L-*threo*-DOPS microinjected into depressor sites of the NTS elicits hypotension and bradycardia without conversion to noradrenaline at doses lower than those of levodopa (Miyamae et al., 1996). These responses are dose-dependent, stereoselective, and DOPA ME-sensitive, but NSD-1015-insensitive. Similar depressor and bradycardic responses to L-*threo*-DOPS are observed in depressor sites of the CVLM (Furukawa et al., 1997). In pressor sites of the RVLM, however, wide dose ranges of microinjected L-*threo*-DOPS expectedly did not elicit hypertension and tachycardia in contrast with levodopa. It is probable that there is an L-*threo*-DOPS-responsive subtype of DOPA recognition site in the hypothalamus, NTS, and CVLM, whereas an L-*threo*-DOPS-unresponsive subtype of the DOPA recognition site exists in the RVLM.

7.4. The striatum and the hippocampal CA1 region

7.4.1. Responses to levodopa via presynaptic β-adrenoceptors and dopamine D_2 receptors in striatal slices

Nanomolar levodopa elicits the propranolol-sensitive facilitation of the release of endogenous dopamine from rat striatal slices evoked by electrical field stimulation via presynaptic β-adrenoceptors under intact AADC activity, and this facilitation is not abolished by an AADC inhibitor (Misu et al., 1986) (Fig. 5A). In contrast, micromolar levodopa elicits the sulpiride-sensitive inhibition of dopamine release via presynaptic dopamine D_2 receptors in the presence of the AADC inhibitor. These responses to levodopa appear to be opposite to those elicited by exogenously applied dopamine. Nanomolar dopamine elicits the sulpiride-sensitive inhibition of the release of endogenous noradrenaline via presynaptic dopamine D_2 receptors, whereas micromolar dopamine elicits the propranolol-sensitive facilitation of the release via presynaptic β-adrenoceptors in hypothalamic slices (Misu et al., 1985). Nanomolar apomorphine also elicits the inhibition of noradrenaline release via presynaptic dopamine D_2 receptors in a manner similar to dopamine. It seems likely that in striata, a primary presynaptic action of levodopa is the facilitation of dopamine release, whereas that of exogenously applied dopamine appears to be inhibition of the release of endogenous dopamine via activation of presynaptic dopamine D_2 autoreceptors.

Some supporting evidence for a presynaptic effect of levodopa in vivo comes from studies with positron emission tomography. By using [^{11}C]levodopa labeled in the β position, the functional tone of the presynaptic dopaminergic system, the turnover of the tracer, can be measured (Tedroff, 1998). Tetrahydrobiopterin, an endogenous cofactor for TH, increases the turnover of [^{11}C]levodopa, i.e., the influx constant for the tracer, in the monkey striatum. This increase is further enhanced by the infusion of tyrosine (Tsukada et al., 1996) and by levodopa (Tedroff et al., 1997). Tyrosine infusion alone does not induce this elevation of radioactivity. It appears likely that the increase in the tracer influx constant elicited by the cofactor for TH and tyrosine is mediated by an increased release of DOPA that affects the presynaptic dopamine neurons (Tedroff, 1998). Indeed, levodopa and exogenously applied apomorphine display opposite effects on the [^{11}C]levodopa influx constant. Levodopa increases this influx constant under the condition of low baseline dopaminergic tone in a dopamine D_2 autoreceptor-antagonist-like manner, whereas apomorphine decreases it under a high baseline tone in a dopamine D_2 autoreceptor-agonist-like manner (Tedroff et al., 1997). These investigators hypothesize that levodopa and dopamine exert divergent influences on presynaptic dopaminergic tone in vivo and that the baseline dopaminergic tone-dependent effects observed following the infusion of levodopa and apomorphine represent a homeostasis-regulating mechanism for dopaminergic turnover in vivo (Tedroff, 1998).

7.4.2. Postsynaptic responses to 3,4-dihydroxyphenylalanine or levodopa and interactions between locomotor activity via postsynaptic dopamine D_2 receptors and basal release of 3,4-dihydroxyphenylalanine in striata

The convincing evidence for a postsynaptic neurophysiological or neuropharmacological response to the release of DOPA or levodopa resides in the effect on locomotor activity. We suggest that DOPA released by nicotine can partially result in nicotine-induced increases in locomotor activity (Nakamura et al., 1993; Goshima et al., 1996a). Nicotine releases DOPA as well as dopamine during striatal

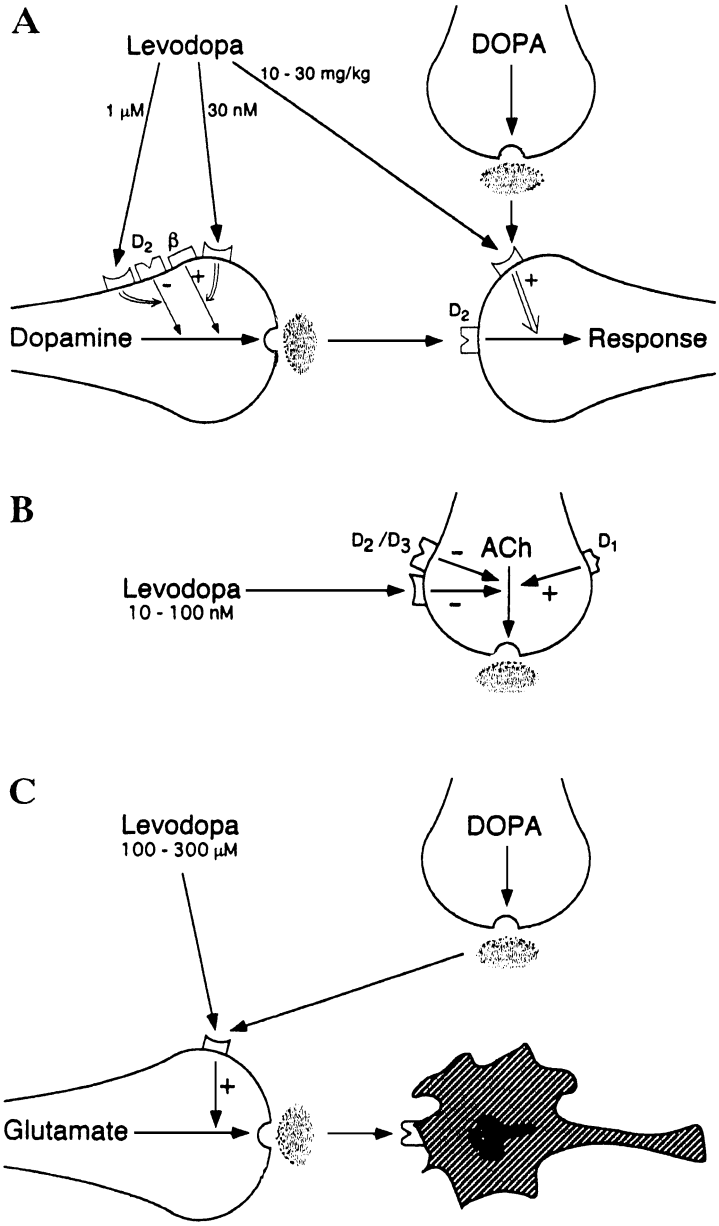

Fig. 5. Responses to levodopa and DOPA released in the rat striatum. Most of the responses to levodopa are stereoselective. In **A**, nanomolar levodopa facilitates the release of endogenous dopamine from striatal slices via activation of presynaptic β-adrenoceptors under intact AADC activity, and this facilitation is not inhibited by a central AADC inhibitor. In contrast, micromolar levodopa inhibits dopamine release via the activation of presynaptic dopamine D_2 receptors under essentially complete inhibition of AADC. Levodopa potentiates, and DOPA release appears to potentiate, the effects of quinpirole, a dopamine D_2 agonist, on locomotion via the activation of postsynaptic dopamine D_2 receptors. Quinpirole-induced locomotor activities are potentiated following an increase in the basal release of DOPA, monitored simultaneously during striatal microdialysis, as a result of AADC inhibition elicited by a central AADC inhibitor, NSD-1015. In contrast, locomotor activities are inhibited following a decrease in the basal release of DOPA as a result of the inhibition of TH elicited by α-MPT. Neither NSD-1015 nor α-MPT alters the decrease in the basal release of dopamine, via the activation of presynaptic dopamine D_2 receptors, elicited by quinpirole alone. In **B**, levodopa inhibits the basal release of ACh in the absence of NSD-1015 during microdialysis in a model of Parkinson's disease. Facilitation of the release of dopamine via presynaptic β-adrenoceptors, potentiation of the activities of postsynaptic dopamine D_2 receptors related to locomotion, and inhibition of the basal release of ACh induced by levodopa itself might increase the effectiveness of levodopa, which is converted to dopamine, in alleviating the symptoms of Parkinson's disease. In **C**, micromolar levodopa induces the vesicular release of glutamate from striatal slices. This release is antagonized by DOPA ME in a competitive manner, but not inhibited by NSD-1015. In primary neuron cultures, micromolar levodopa elicits auto-oxidation-independent neuronal death as a result of glutamate release in the absence of NSD-1015. Four-vessel occlusion elicits the release of DOPA, dopamine, and glutamate during microdialysis. DOPA released upstream appears to cause the release of glutamate, which then leads to delayed neuronal death. Intrastriatal perfusion of NSD-1015 increases the basal release of DOPA following inhibition of AADC, facilitates glutamate release, and exaggerates delayed neuronal death induced by ischemia. In contrast, intrastriatal perfusion of nanomolar DOPA CHE, a most potent and relatively stable competitive antagonist for levodopa among DOPA ester compounds, antagonizes DOPA-induced glutamate release and delayed neuronal death by ischemia. NSD-1015 tends to reduce dopamine release evoked by ischemia, and DOPA CHE elicits no effect on it. Modified from Misu et al. (2002b).

microdialysis (Nakamura et al., 1992b). However, it seems likely that DOPA release, but not dopamine release, is tonically regulated via the activation of central nicotinic ACh receptors because of the fact that local perfusion of the antagonist mecamylamine inhibits the basal release of DOPA, but not that of dopamine. The subcutaneous injection of mecamylamine (2 mg/kg) also appears to preferentially inhibit DOPA release rather than dopamine release evoked by electrical foot-shock in the nucleus accumbens (Yamanashi et al., 2001). In addition, the intraperitoneal injection of a usual concentration of α-MPT (200 mg/kg), to inhibit TH activity (Nakamura et al., 1992a; Yue et al., 1993a, 1993b, 1994a, 1994b), abolishes the basal release of both DOPA and dopamine during striatal microdialysis (Nakamura et al., 1992a). However, it is an interesting finding that a far lower concentration of the inhibitor (3 mg/kg) decreases basal DOPA release by one-third without any tendency of a decrease in basal dopamine release (Nakamura et al., 1993). This selective low concentration of α-MPT inhibits mecamylamine-sensitive increases in locomotor activity elicited by the subcutaneous injection of nicotine (0.4 mg/kg).

Both the release of DOPA (Nakamura et al., 1993) and levodopa (Nakamura et al., 1994) appear to increase locomotor activity. In intact rats pretreated with an intraperitoneal injection of NSD-1015 (100 mg/kg), the intraperitoneal injection of levodopa (100 mg/kg) markedly increases locomotor activity (Nakamura et al., 1994). This is consistent with the other findings of motor responses to levodopa (200 mg/kg) in normal rats (Nakazato & Akiyama, 1989) and of those to levodopa (200 mg/kg) in reserpinized rats in the presence of benserazide, a peripheral AADC inhibitor (Fisher et al., 2000). The effect of levodopa (Nakamura et al., 1994) appears to be postsynaptic in nature because a non-effective dose of levodopa in intact rats (30 mg/kg) elicits marked increases in locomotor activity following the nigrostriatal dopaminergic lesion with the intracerebroventricular injection of 6-hydroxydopamine. This is in part consistent with the other findings of Treseder et al. (2001). In unilateral 6-hydroxydopamine-lesioned rats, the oral application of NSD-1015 (100 mg/kg) increases the total counts of contralateral circling induced by the intraperitoneal injection of levodopa (25 mg/kg) and carbidopa (12.5 mg/kg), a peripheral AADC inhibitor (Treseder et al., 2001). NSD-1015 (50–150 mg/kg), however, elicits a dose-dependent delay of the onset of rotations, as well as a decrease in the rate of rotations. Mechanisms of levodopa-induced increases in motor activity should be further explored. However, the non-effective doses of levodopa (30 mg/kg in intact rats and 10 mg/kg in lesioned rats) potentiate increases in locomotor activity elicited by the subcutaneous injection of quinpirole (0.1–1 mg/kg), a dopamine D_2 agonist, in the presence of AADC inhibition (Nakamura et al., 1994) (Fig. 5A). This potentiation by levodopa appears to be independent of the traditionally believed bioconversion to dopamine. The simultaneously monitored basal release of both DOPA and dopamine during striatal microdialysis in the intact rat suggests that the potentiation occurs following increases in basal DOPA release and decreases in basal dopamine release. Pretreatment with NSD-1015 alone increases basal DOPA release for the initial 40 min, which is followed by a further increase as a result of the simultaneous addition of levodopa (30 mg/kg) and quinpirole (1 mg/kg) compared with saline and quinpirole. In contrast, pretreatment with NSD-1015 alone elicits either no change in basal dopamine release until 120 min after application (Nakamura et al., 1992a) or a decrease in release in the first 40 min following application (Nakamura et al., 1994). These disparate results probably are due to differences in experimental conditions. In the latter study (Nakamura et al., 1994), the NSD-1015-induced initial decrease in basal dopamine release is followed by a further decrease as a result of the addition of quinpirole and saline, probably via activation of presynaptic dopamine D_2 receptors, and possibly by AADC inhibition. This further decrease in basal dopamine release is not affected by the addition of quinpirole and levodopa.

Furthermore, it seems likely that basally released DOPA functions to potentiate increases in locomotor activity elicited by quinpirole (Yue et al., 1994a) (Fig. 5A). Quinpirole alone (1 mg/kg) increases locomotor activity in intact rats via the activation of postsynaptic dopamine D_2 receptors (Nakamura et al., 1994) and also decreases the basal release of both DOPA and dopamine, probably via the activation of presynaptic dopamine D_2 receptors. Increases in locomotor activity induced by quinpirole alone are inhibited following further decreases in basal DOPA release elicited by the prior intraperitoneal injection of the selective low concentration of α-MPT (3 mg/kg), which decreases basal DOPA release by one-third without any tendency of a decrease in basal dopamine release (Nakamura et al., 1993). In contrast, increases in locomotor activity by quinpirole alone are potentiated following opposite increases in basal DOPA release elicited by NSD-1015 (100 mg/kg) as a result of AADC inhibition (Yue et al., 1994a). NSD-1015 alone markedly increases basal DOPA release (Nakamura et al., 1992a; Furukawa et al., 2001), which appears to function by itself. This effect of DOPA appears to be independent of the traditionally believed bioconversion to dopamine because neither α-MPT (3 mg/kg) nor NSD-1015 (100 mg/kg) alters the decrease in basal dopamine release elicited by quinpirole alone. These findings of levodopa- and DOPA-induced potentiation of increases in locomotor activity elicited by quinpirole suggest the existence of some mutual synergistic interaction between postsynaptic recognition sites of DOPA and postsynaptic dopamine D_2 receptors (Nakamura et al., 1994; Yue et al. 1994a).

Supporting evidence for these findings comes from in vivo studies with positron emission tomography and with a labeled dopamine D_2 ligand. Following the acute administration of levodopa under inhibition of central AADC, the binding of [^{11}C]raclopride, an indicator for dopamine D_2

receptor density, was shown to increase (Hume et al., 1995; Opacka-Juffry and Brooks, 1995). A single administration of levodopa has been shown to increase the number of dopamine D_2-binding sites by using tritiated spiperone in the absence of NSD-1015 (Murata and Kanazawa, 1993).

On the other hand, completely opposite findings have been reported regarding the effects of NSD-1015 on levodopa-induced motor activity. NSD-1015 (100 mg/kg) inhibits the contralateral circling response to levodopa (10 mg/kg) in 6-hydroxydopamine-lesioned mice (Goodale and Moore, 1976). NSD-1015 (100 mg/kg) elicits a delay in the onset and a decrease in the rate of circling induced by levodopa (50 mg/kg), although this motor response is observed in < 50% of rats (Melamed et al., 1984). Treseder et al. (2000) showed that in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated common marmosets, pretreatment with intraperitoneally injected NSD-1015 (10–50 mg/kg) worsened their baseline motor deficits. This pretreatment abolished both increases in locomotor activity and reversal of disability elicited by the subcutaneous injection of levodopa (5–18 mg/kg). NSD-1015 (25 mg/kg) abolished the increase in locomotor activity and the improvement in disability elicited by a dopamine D_1 agonist or quinpirole.

The discrepancies between the findings obtained by Goodale and Moore (1976), Melamed et al. (1984), and Treseder et al. (2000, 2001) and by Nakazato and Akiyama (1989), Nakamura et al. (1994), Yue et al. (1994a), and Fisher et al. (2000) appear to reflect the dual personalities of levodopa and released DOPA. These personalities encompass (1) the traditionally established bioconversion of levodopa to dopamine (Carlsson et al., 1957; Bartholini et al., 1967; Hefti and Melamed, 1980) and (2) the role of levodopa itself as a probable neurotransmitter and/or neuromodulator. In this respect, we have introduced reasonable experimental approaches. The responses to levodopa we observed were primarily obtained in the absence, as well as the presence, of a central AADC inhibitor. An example of these responses is the facilitation of noradrenaline and dopamine release via the activation of presynaptic β-adrenoceptors from rat hypothalamic and striatal slices (Goshima et al., 1986; Misu et al., 1986). The levodopa-induced propranolol-sensitive facilitation of dopamine release was also evident in the absence of NSD-1015 in striatal slices isolated from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57 black mice (Goshima et al., 1991a), an animal model for Parkinson's disease (Arai et al., 1990). Another example is the glutamate release from striatal slices (Goshima et al., 1993) and the inhibition of population spikes in the hippocampal CA1 region (Akbar et al., 2001). The same evidence is also obtained in the depressor and bradycardic responses in the NTS (Kubo et al., 1992; Misu et al., 1997; Furukawa et al., 2000) and the CVLM (Yue et al., 1993a), and the pressor and tachycardic responses in the RVLM (Yue et al., 1993b). Furthermore, we have proved the antagonism of responses to levodopa or DOPA released by the competitive antagonists DOPA ME (Goshima et al., 1991b, 1991c, 1993; Kubo et al., 1992; Yue et al., 1993a, 1993b) and DOPA CHE (Misu et al., 1997; Furukawa et al., 2000, 2001; Akbar et al., 2001).

In the case of increases in locomotor activity elicited by nicotine (Nakamura et al., 1993), levodopa (Nakamura et al., 1994), and DOPA released (Yue et al., 1994a), we could not use DOPA ME at that time because this antagonist is readily hydrolyzed to DOPA. Nicotine-induced increases in locomotor activity are not antagonized, but potentiated by pretreatment with intracerebroventricularly injected DOPA ME (Nakamura et al., 1993). Thus, we have to find potent, peripherally or orally effective, and stable antagonists of levodopa and released DOPA.

7.4.3. Levodopa-induced inhibition of the basal release of acetylcholine in rat models of Parkinson's disease

Rats given a nigrostriatal dopaminergic hemilesion with 6-hydroxydopamine prior to local injection of levodopa (10–100 nM) into the medial forebrain bundle, in the absence of NSD-1015, experienced an inhibition of the basal release of ACh during striatal microdialysis compared with sham-operated rats (Ueda et al., 1995) (Fig. 5B). This inhibition elicited by levodopa is independent of the conversion of dopamine from levodopa because dopamine (100 nM) administered at the same dose to the highest dose of levodopa elicits no inhibition. The levodopa-induced inhibition of the basal release of ACh is not antagonized by sulpiride, a dopamine D_2/D_3 antagonist, whereas selective dopamine D_2 and D_3 agonists inhibit the basal release of ACh in a completely sulpiride-sensitive manner (Sato et al., 1994a). In addition, a selective dopamine D_1 agonist oppositely increases the basal release of ACh in a dopamine D_1-antagonist-sensitive manner (Sato et al., 1994b).

7.4.4. Supplemental effects of levodopa itself on alleviating the symptoms of Parkinson's disease

As mentioned in Sections 7.4.1–7.4.3, levodopa itself facilitates dopamine release via the activation of presynaptic β-adrenoceptors (Misu et al., 1986; Goshima et al., 1991a), potentiates the activity of postsynaptic dopamine D_2 receptors related to locomotion (Nakamura et al., 1994), and inhibits the basal release of ACh (Ueda et al., 1995). All of these responses to levodopa itself might further increase its effectiveness in alleviating the symptoms of Parkinson's disease, especially at the early stages (Misu et al., 1996, 2002b).

7.4.5. Levodopa releases vesicular glutamate from in vitro and in vivo striata

Levodopa causes the release of endogenous glutamate from striatal slices with an ED_{50} value of 140 μM (Goshima et al., 1993) in a partially TTX-sensitive and Ca^{2+}-dependent manner, which suggests, at least in part, vesicular release of glutamate (Fig. 5C). This release is elicited by levodopa itself because it is also observed under inhibition

of AADC and is antagonized by DOPA ME in a competitive manner. This effect of levodopa is independent of dopamine because dopamine elicits no release. Micromolar levodopa also releases glutamate from cultured striatal neurons in the absence of NSD-1015 (Maeda et al., 1997). Intracellular signal transduction mechanisms for some excitatory effects of levodopa are not clear at present. In Meissner's plexus preparations of guinea-pigs, however, nanomolar levodopa increases by itself the amplitude of excitatory postsynaptic potentials without increasing the sensitivity to exogenously applied ACh. In addition, it augments the intracellular Ca^{2+} concentration as measured by microfluorophotometry with fura-2 (Hirai et al., 1996).

We observed a surprising phenomenon during striatal microdialysis of conscious rats, i.e., that levodopa at 30–100 nM causes the release of glutamate. Additionally, a non-effective dose of levodopa (10 nM) in intact animals markedly increases glutamate release in rats that received a nigrostriatal dopaminergic lesion by prior intracerebroventricular injection of 6-hydroxydopamine (Okumura et al., 1995; Goshima et al., 1996b; Misu et al., 1996). Unfortunately, however, we could not reconfirm these findings, although exact reasons are not clear. Finally, we confirmed that micromolar levodopa stereoselectively releases glutamate in the absence of NSD-1015 during striatal microdialysis of intact rats (Furukawa et al., 2001). Levodopa-induced glutamate release appears to be involved in neuroexcitatory side effects like the dyskinesia encountered during chronic therapy of Parkinson's disease (Greenamyre, 1993; Misu et al., 1996). There has been a concern that the chronic administration of levodopa might accelerate dopaminergic neurodegeneration, particularly at progressive stages of Parkinson's disease (Blunt et al., 1993; Misu et al., 1996).

7.4.6. The hippocampal CA1 region

In the hippocampal CA1 region, nanomolar levodopa inhibits population spikes by electrical stimuli applied to the Schaffer collateral/commissural fibers (Akbar et al., 2001). This inhibition is also seen in the presence of NSD-1015 and is antagonized by DOPA CHE. Levodopa appears to release noradrenaline, which, in turn, releases GABA via α-adrenoceptors on GABA neurons, thereby inhibiting population spikes via activation of $GABA_A$ receptors.

7.4.7. 3,4-Dihydroxyphenylalanine and neuronal death in the striatum and the hippocampal CA1 region

7.4.7.1. Preface. Levodopa causes neuronal death mainly via three cascades: (1) auto-oxidation to yield neurotoxic hydroxyl radicals (Cheng et al., 1996; Fahn, 1996); (2) auto-oxidation to yield 3,4,6-trihydroxyphenylalanine (Fig. 1) and its quinone derivative, a non-NMDA agonist, and excitotoxin (Olney et al., 1990; Newcomer et al., 1995); and (3) the release of glutamate (Maeda et al., 1997; Furukawa et al., 2001; Arai et al., 2001) in a TTX-sensitive and Ca^{2+}-dependent manner (Goshima et al., 1993). Excitotoxicity that results from increased extracellular glutamate elicits neuronal death (Olney & Sharpe, 1969; Choi, 1988; Obrenovitch & Richards, 1995), and is implicated in a final common pathway for neurologic disorders (Choi, 1988; Lipton & Rosenberg, 1994).

7.4.7.2. Cultured striatal neurons. Cultured striatal neurons are protected by ascorbic acid, an antioxidant, against auto-oxidation and death elicited by both levodopa and dextrodopa, in 3 days in culture, and by dopamine, in 3 and 10 days in culture (Cheng et al., 1996). Furthermore, levodopa elicits both stereoselective and antioxidant-insensitive neurotoxicity in 10 days in culture (Fig. 5C). The application of TTX, Ca^{2+} omission, and Mg^{2+} addition protects against this kind of neuronal death (Maeda et al., 1997). The antagonist of the NMDA receptor ion channel (+)-5-methyl-10,11-dihydro-5*H*-dibenzol[*a*,*d*]cyclohepten-5,10-imine maleate (MK-801) (Choi, 1988; Cheng et al., 1996) and a non-NMDA antagonist (Sheardown et al., 1990; Cheng et al., 1996) antagonize neuronal death that results from glutamate release (Maeda et al., 1997). It appears to be mediated by vesicular release of glutamate (Goshima et al., 1993). Glutamate can elicit Ca^{2+} influx via its receptors (Choi, 1988; Sheardown et al., 1990), which can lead to activation of nNOS and the production of neurotoxic NO (Dawson et al., 1993).

7.4.7.3. 3,4,-Dihydroxyphenylalanine is an upstream factor for glutamate release and delayed neuronal death by transient ischemia in the striatum and the hippocampal CA1 region. In the striatum, 10-min 4-vessel occlusion releases DOPA, dopamine, and glutamate during microdialysis and elicits mild neuronal death 4 days after reperfusion (Furukawa et al., 2001) (Fig. 5C). The DOPA released appears to cause release of glutamate and delayed neuronal death elicited by ischemia. Indeed, intrastriatal perfusion of NSD-1015 (30 μM) increases the basal release of DOPA as a result of AADC inhibition, enhances release of glutamate, and exaggerates delayed neuronal death by ischemia. In contrast, intrastriatal perfusion of nanomolar DOPA CHE (10–100 nM) antagonizes release of glutamate and protects delayed neuronal death by ischemia. The dopamine released appears not to be involved in the release of glutamate elicited by ischemia, because NSD-1015 tends to reduce the increase in dopamine release evoked by ischemia and DOPA CHE had no effect on it.

The hippocampal CA1 region is highly vulnerable to the effects of ischemia (Choi, 1988; Obrenovitch & Richards, 1995). Ischemia of 5-min and 10-min duration elicits a slight and mild release of glutamate during microdialysis and then mild and severe delayed neuronal death, respectively (Arai et al., 2001). These events elicited by 5-min ischemia are abolished by intrahippocampal perfusion of nanomolar DOPA CHE (100 nM), probably via antagonism of the effects of DOPA released. However, the release of DOPA is below assay sensitivity in this design. DOPA

release that is less than the measurable extracellular level (0.4 nM) (Furukawa et al., 2001) appears to cause release of glutamate and delayed neuronal death elicited by ischemia because extremely low concentrations of levodopa (3–10 pM) exert responses that potentiate activities of presynaptic β-adrenoceptors (Goshima et al., 1991c). These findings suggest that the sensitivity of a biochemical analysis used, the release of DOPA, to find DOPAergic components to be identified as a neurotransmitter and/or neuromodulator is lower compared with pharmacological approaches by means of DOPAergic agonists and antagonists.

Although DOPA CHE acts on the ion channel of NMDA receptors with millimolar IC_{50} (Miyamae et al., 1999a), this site probably is unrelated to the protective effect of DOPA CHE because a nanomolar dose of DOPA CHE is effective. Protection elicited by systemic MK-801 is attributed largely to hypothermia (Buchan & Pulsinelli, 1990). DOPA CHE, however, does not cause hypothermia, at least following intrastriatal perfusion (Furukawa et al., 2001).

In contrast, DOPA CHE does not inhibit an increase in glutamate elicited by 10-min ischemia. Different mechanisms might underlie the effects of mild and severe ischemia. We suggest that glutamate released by mild ischemia is largely vesicular, but the glutamate increase elicited by severe ischemia is mainly mediated by the reversed operation of neuronal glutamate transporters (Rossi et al., 2000). DOPA might not be involved in this process.

8. Conclusion

Traditionally, DOPA has been believed to be nothing but a precursor for dopamine. Since the review by Misu et al. (1996), we have further provided evidence to suggest that DOPA itself fulfills the criteria for neurotransmitters and/or neuromodulators, including synthesis, metabolism, active transport, presence, physiological release, competitive antagonism, and responses, in addition to being the precursor of dopamine. DOPA appears to be a neurotransmitter of the primary baroreceptor afferents terminating in the NTS. Glutamate has been regarded as the most probable neurotransmitter. However, we propose a new pathway in which DOPA is a neurotransmitter of the primary ADN and glutamate is a neurotransmitter of the second-order neurons in the NTS. Levodopa might release undetectable, but functioning, glutamate from the secondary neurons via DOPA recognition sites. In addition, levodopa releases vesicular glutamate from striata to elicit auto-oxidation-independent neuronal death. DOPA released by four-vessel occlusion appears to be an upstream factor for glutamate release and delayed neuronal death. The common sequence of events leading to baroreflex neurotransmission and delayed neuronal death appears to be DOPA-induced glutamate release, the activation of ionotropic glutamate receptors, nNOS activation, and NO production. However, the interactions between the released glutamate and compounds produced as a result of DOPA auto-oxidation might start amplifying cycles of neurotoxic cascades. Thus, levodopa therapy might accelerate neuronal degeneration, particularly at progressive stages of Parkinson's disease.

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