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α_2-Adrenoceptor modulation of 5-HT biosynthesis in the rat brain

Mitsuhiro Yoshioka[a], Machiko Matsumoto[a], Hiroko Togashi[a], Charles B. Smith[b] and Hideya Saito[a]

[a]*First Department of Pharmacology, Hokkaido University School of Medicine, Sapporo (Japan) and* [b]*Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, MI 48109-0626 (USA)*

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The purpose of the present study is to clarify the modulation of the biosynthesis of serotonin (5-HT) *via* the α_2-adrenoceptors in the brain. For this purpose, 5-hydroxytryptophan (5-HTP) accumulation was determined using an HPLC-ECD system in the presence of the inhibition of aromatic L-amino acid decarboxylase. Administration of α_2-adrenoceptor agonist, clonidine, produced a reduction of the in vivo 5-HTP accumulation in the rat hippocampus and dorsal raphe nucleus. In addition, α_2-adrenoceptor antagonist, idazoxan, increased the 5-HTP accumulation in both the hippocampus and the dorsal raphe nucleus. In rats with catecholaminergic neurons denervated by pretreatment with 6-hydroxydopamine, clonidine failed to produce a reduction of 5-HTP accumulation in the dorsal raphe nucleus. On the other hand, hippocampal 5-HTP accumulation was decreased significantly. Brain tryptophan levels were unaffected by either clonidine or idazoxan. These results suggest that α_2-adrenoceptors might modulate serotonin biosynthesis and this modulation might be related to the neuroanatomical differences in the rat brain.

Electrical stimulation of the serotonergic neurons in the CNS increases not only serotonin (5-HT) turnover [20], but also 5-HT synthesis by the activation of tryptophan hydroxylase [4, 11], which is the initial and rate-limiting enzyme in the biosynthesis of 5-HT. A 5-HT_{1A} agonist, 8-OH-DPAT, potently suppresses the firing rate of 5-HT neurons [21] and reduces 5-hydroxytryptophan (5-HTP) accumulation [8] in the rat brain. Sawada and Nagatsu [19] also reported that stimulation of the 5-HT autoreceptors prevents the increase of 5-HT biosynthesis in rat raphe slices.

Anatomical studies suggest that the serotonergic neurons of the midbrain raphe nuclei are innervated by norepinephrinergic nerve terminals [2]. The firing activity of the 5-HT neurons of the rat dorsal raphe nucleus is modulated by α-adrenoceptor agonists and antagonists [3, 23]. In addition, hippocampal formation was considerably innervated by both serotonergic and norepinephrinergic fibers [14, 16]. In hippocampal slices, electrical stimulation-induced [^{3}H]5-HT release is inhibited via α_2-adrenoceptor stimulation [9]. Reinhard and Roth [18] reported that the systemic administration of clonidine decreased the brain cortex concentration of 5-hydoxyindoleacetic acid (5-HIAA), a major metabolite of 5-HT, and suggested that this inhibitory effect was mediated by decreased 5-HT release. It is conceivable, therefore, that transmitter biosynthesis may also be modulated by norepinephrine released from neighboring norepinephrinergic nerve endings in both the cell body and the nerve terminals. As with the above findings on clonidine, the neuroanatomical difference of interaction between serotonergic and norepinephrinergic neurons must also be considered in terms of 5-HT synthesis, i.e., 5-HT cell body and 5-HT nerve terminal. In the present study, we focused on neuroanatomical differences and investigated direct and/or indirect actions of α_2-adrenoceptor-mediated 5-HT synthesis in the rat brain.

Male Wistar rats (150–180 g) were obtained from the Shizuoka Laboratory Animals Center (Hamamatsu, Japan). The daily light period was set between 07.00–19.00 h. Groups of 4–6 rats received an intraperitoneal injection of clonidine, idazoxan or saline. Thirty min thereafter, all rats received 3-hydroxybenzylhydrazine (NSD-1015, an inhibitor of aromatic L-amino acid decarboxylase, 100 mg/kg i.p.) and were decapitated 30 min later [5].

Brains were rapidly removed and dissected on an ice-cold plate to separate the hippocampus, the dorsal raphe nucleus and the rest of the brain [10, 17]. The slices were disrupted by sonication with a 5 to 10 times volume of 0.4 N perchloric acid containing 10 μM EDTA-2Na and *N*-methyldopamine as an internal standard. Precipitated

Correspondence: M. Yoshioka, First Department of Pharmacology, Hokkaido University School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060, Japan. Fax: (81) 11-717-5286.

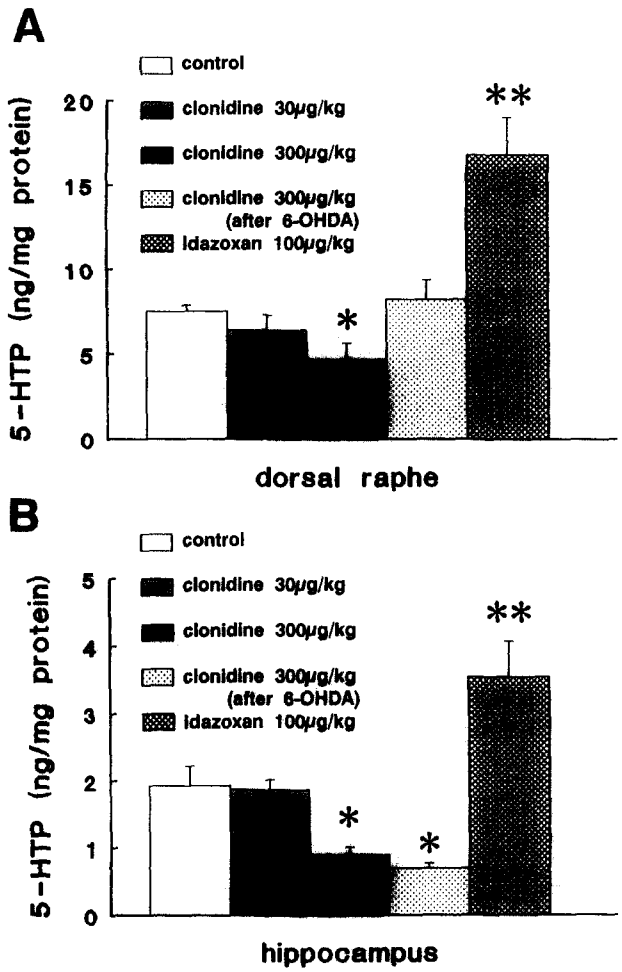

Fig. 1. Effects of clonidine and idazoxan on the formation of 5-HTP in the dorsal raphe nucleus (A) and in the hippocampus (B). Each column and bar represents mean ± S.E.M. values from 4–6 different experiments. * and ** indicate significant differences ($P<0.05$ and 0.01, respectively) from the saline control values.

protein was removed by centrifugation at -2°C at 10,000 rpm for 10 min.

For the determination of 5-HTP in the hippocampus and the dorsal raphe nucleus, and the tryptophan in the rest of the brain, 5–50 μl of supernatant was injected into a high-performance liquid chromatograph (HPLC) apparatus consisting of a Yanagimoto VMD-101 pump, a reversed-phase column (RP-18, ODS, 5 μm particle size) and an electrochemical detector (ECD-100, Eicom, Kyoto, Japan). Applied potential for determination of 5-HTP and tryptophan was maintained at 700 mV and 900 mV vs Ag/AgCl, respectively. Catecholamines (norepinephrine and dopamine) in the rest of the brain were assayed by alumina extraction after deproteinization and measured by HPLC-ECD [15].

In order to destroy norepinephrinergic neurons, some rats were injected i.c.v. with 6-hydroxydopamine (6-OHDA, 200 μg) 10 min after the administration of pargyline (25 mg/kg, i.p.) to prevent the destruction of serotonergic neurons. The administration of the neurotoxin was carried out with a microsyringe that was positioned with a stereotaxic apparatus at a depth of 3.3 mm below the surface of the dura mater, 1.4 mm lateral to the midline and 0.8 mm posterior to the bregma. All i.c.v. injections were made in a volume of 10 μl and delivered over a period of 2 min. Measurements were taken 14 days after the i.c.v. injection of 6-OHDA.

The following drugs were used: clonidine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), idazoxan hydrochloride (Reckitt And Colman, Hull, UK), 3-hydroxybenzylhydrazine (NSD-1015, Sigma), 6-OHDA (Sigma) and pargyline (Sigma). Data were analyzed by Dunnett's multiple range test or analysis of variance followed by the Student's *t*-test.

The saline control values of 5-HTP accumulation were 7.5±0.4 (mean±S.E.M.) and 1.9±0.3 ng/mg protein in the dorsal raphe nucleus and the hippocampus, respectively. Clonidine (300 μg/kg, i.p.) produced a significant reduction of this 5-HTP accumulation in both the hippocampus and the dorsal raphe nucleus (4.7±0.9 and 0.9±0.1 ng/mg protein, respectively; Fig. 1). A low dose of clonidine (30 μg/kg) decreased the content of 5-HTP slightly in the dorsal raphe nucleus but not significantly in either the hippocampus or the dorsal raphe nucleus. In order to determine whether clonidine was acting directly upon 5-HT neurons or indirectly through norepinephrinergic neurons, clonidine (300 μg/kg, i./p.) was injected into rats which had been pretreated with 6-OHDA to destroy their norepinephrinergic neurons. The data in Table I reveal that 6-OHDA reduced NE by greater than 80% and DA by approximately 77% without affecting the level of tryptophan (Table II). The treatment with 6-OHDA prevented clonidine-induced 5-HTP accumulation in the dorsal raphe nucleus (8.2±1.1 ng/mg protein; n.s. vs. control). In the hippocampus pretreated with 6-OHDA, clonidine still decreased 5-HTP accumulation

TABLE I

EFFECT OF 6-OHDA ON CATECHOLAMINE CONCENTRATIONS IN THE REST OF THE BRAIN

NE, norepinephrine; DA, dopamine. All values are mean ± S.E.M. and expressed as ng/g wet weight.

	control (5)	6-OHDA (4)
NE	242.1 ± 5.7	46.6 ± 20.0**
DA	535.1 ± 14.6	124.2 ± 58.3*

* and ** express significant differences between the control values ($P<0.01$ and $P<0.001$, respectively).

(0.6±0.1 ng/mg protein; $P<0.05$ vs. control) and this reduction was greater than that in the group not treated with 6-OHDA (Fig. 1).

In addition, an α_2-adrenoceptor antagonist, idazoxan (100 μg/kg, i.p.), produced increases in 5-HTP accumulation in both the dorsal raphe nucleus (16.8 ng/mg protein; $P<0.01$ vs. control) and the hippocampus (3.6 ng/mg protein; $P<0.01$ vs. control). As shown in Table II, these drugs showed no effects on the brain tryptophan level.

Clonidine (300 μg/kg, i.p.) produced a reduction of 5-HTP accumulation in both the dorsal raphe nucleus and the hippocampus by approximately 40% and 50%, respectively, under aromatic L-amino acid decarboxylase inhibition. The present data are in agreement with those of Reinhard and Roth [18] who observed that clonidine administration decreased 5-HTP accumulation in the rat cerebral cortex by approximately 40%. The distribution of norepinephrinergic and serotonergic fibers appears to overlap considerably in the hippocampus [14, 16]. The possibility that 5-HT biosynthesis is directly modulated via α_2-adrenoceptors is supported by the facts that α_2-adrenoceptor antagonist, idazoxan, produced an increase in 5-HTP accumulation and that pretreatment with 6-OHDA did not affect the inhibitory effect of intraperitoneally administered clonidine. In contrast to its effect in the hippocampus, the inhibitory effect of clonidine on the 5-HT neurons in the dorsal raphe nucleus is probably indirect. This view is supported by the fact that pretreatment with 6-OHDA eliminated the inhibitory effect of intraperitoneally administered clonidine. Assuming that this dose of 6-OHDA selectively destroys catecholaminergic neurons, the inhibitory effect of clonidine in the dorsal raphe might require mediation by an intact NE system.

A lower dose of clonidine (30 μg/kg, i.p.) failed to produce a reduction of 5-HTP accumulation in either the hippocampus or the dorsal raphe nucleus. Although 30 μg/kg of clonidine decreases blood pressure in rats [24], this dose was insensitive to 5-HT synthesis in the present study. The possibility that high doses of clonidine suppress 5-HTP accumulation due to a systemic effect (e.g. hypotension) can be excluded because, as Togashi [24] reported, the hypotensive effect of clonidine at a dose of 30 μg/kg is saturated, and there is no significant difference in systolic blood pressure changes between 30 and 300 μg/kg. In addition, Echizen and Freed [6] reported that during hypotension induced by nitroprusside, extracellular concentrations of NE and 5-HIAA in the rat dorsal raphe nucleus did not change significantly. Furthermore, the α_2-adrenoceptor antagonist, idazoxan (100 μg/kg, i.p.), produced an effect opposite to that of clonidine; it facilitated 5-HTP accumulation. Taken together, these data indicate that the modulation of 5-HTP accumulation is not mediated by the non-specific systemic effect of clonidine but by the specific α_2-adrenoceptors.

The rate of 5-HT synthesis in the brain is sensitive to changes in the concentration of its substrate, tryptophan [1, 5, 7]. The observation in the present study that clonidine or idazoxan did not alter tryptophan levels suggests that the action of these drugs on 5-HTP accumulation are not due to changes in the substrate availability.

It was reported that tryptophan hydroxylase activity was controlled by Ca^{2+} [13] and that this effect of calcium on the activity of tryptophan hydroxylase was mediated by the calcium-calmodulin-dependent phosphorylation of tryptophan hydroxylase [12, 26]. Although the mechanism by which α_2-adrenoceptor stimulation inhibits neurotransmitter release has not been established, the influence on the availability of intracellular calcium to promote exocytotic release is probably involved [22, 25]. It is presumed that α_2-adrenoceptor-mediated inhibition of tryptophan hydroxylase activity is modulated by the same mechanism, i.e., the reduction of calcium availability in nerve terminals and cell bodies. However, the precise mechanism of this action is uncertain.

In conclusion, it is hypothesized that α_2-adrenoceptor activation inhibits 5-HT synthesis at the tryptophan hydroxylase level via prejunctional 5-HT heteroreceptors in the hippocampus and through certain catecholaminergic neurons in the dorsal raphe nucleus.

TABLE II

EFFECTS OF CLONIDINE AND IDAZOXAN ON BRAIN TRYPTOPHAN LEVEL

All values are mean ± S.E.M. from 4–6 different experiments and are expressed as nmol/g.

Saline	Clonidine (μg/kg)			Idazoxan (μg/kg)
	30	300	300*	100
10.5 ± 1.2	12.9 ± 0.9	10.9 ± 1.5	9.8 ± 0.6	11.1 ± 0.9

*indicates the 6-OHDA (200 μg, i.c.v.)-treated group.

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1 Ashcroft, G.W., Eccleston, D. and Crawford, T.B.B., 5-Hydroxyindole metabolism in rat brain. A study of intermediate metabolism using the technique of tryptophan loading-I, J. Neurochem., 12 (1965) 483–492.

2 Baraban, J.M. and Aghajanian, G.K., EM autoradiographic studies of adrenergic input to the dorsal raphe, Soc. Neurosci. Abstr., (1979) 329.

3 Baraban, J.M. and Aghajanian, G.K., Suppression on firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor an-

tagonists, Neuropharmacology, 19 (1980) 355–363.
4 Boadle-Biber, M.C., Johannessen, J.N., Narasimhachari, N. and Phan, T.-H., Activation of tryptophan hydroxylase by stimulation of central serotonergic neurons, Biochem. Pharmacol., 32 (1983) 185–188.
5 Carlsson, A. and Lindqvist, M., Effects of antidepressant agents on the synthesis of brain monoamines, J. Neural Transm., 43 (1978) 73–91.
6 Echizen, H. and Freed, C.R., Altered serotonin and norepinephrine metabolism in rat dorsal raphe nucleus after drug-induced hypertension, Life Sci., 34 (1984) 1581–1589.
7 Fernstrom, J.D. and Wurtman, R.J., Brain serotonin: physiological dependence on plasma tryptophan levels, Science, 173 (1971) 149–152.
8 Fernstrom, M.H., Massoudi, M.S. and Fernstrom, J.D., Effect of 8-hydroxy-2-(di-*N*-propylamino)-tetralin on the tryptophan-induced increase in 5-hydroxytryptophan accumulation in rat brain, Life Sci., 47 (1990) 283–289.
9 Frankhuyzen, A.L. and Mulder, A.H., Noradrenaline inhibits depolarization-induced ^{3}H-serotonin release from slices of rat hippocampus, Eur. J. Pharmacol., 63 (1980) 179–182.
10 Glowinski, J. and Iverson, L.L., Regional studies of catecholamines in the rat brain. I. The disposition of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-dopa in various regions of the brain, J. Neurochem., 13 (1966) 655–669.
11 Herr, B.E., Gallager, D.W. and Roth, R.H., Tryptophan hydroxylase: activation in vivo following stimulation of central serotonergic neurons, Biochem. Pharmacol., 33 (1975) 1031–1042.
12 Kuhn, D.M., O'Callaghan, J.P., Juskevich, J. and Lovenberg, W., Activation of brain tryptophan hydroxylase by ATP-Mg: dependence on calmodulin, Proc. Natl. Acad. Sci. USA, 77 (1980) 4688–4691.
13 Kuhn, D.M., Vogel, R.L. and Lovenberg, W., Calcium-dependent activation of tryptophan hydroxylase by ATP and magnesium, Biochem. Biophys. Res. Commun., 82 (1978) 759–766.
14 Loy, R., Koziell, D.A., Lindsey, J.D. and Moore, R.Y., Noradrenergic innervation of the adult rat hippocampal formation, J. Comp. Neurol., 189 (1980) 699–710.
15 Matsumoto, M., Togashi, H., Yoshioka, M., Hirokami, M., Morii, K. and Saito, H., Simultaneous high-performance liquid chromatographic determination of norepinephrine, serotonin, acetylcholine and their metabolites in the cerebrospinal fluid of anesthetized normotensive rats, J. Chromatogr., 526 (1990) 1–10.
16 Moore, R.Y. and Halaris, A.E., Hippocampal innervation by serotonin neurons of the midbrain raphe in the rat, J. Comp. Neurol., 164 (1975) 171–184.
17 Paxinos, S. and Watson C., The Rat Brain in Stereotaxic Co-ordinates, 2nd edn., Academic Press, New York, 1986.
18 Reinhard, J.F. and Roth, R.H., Noradrenergic modulation of serotonin synthesis and metabolism. I. Inhibition by clonidine in vivo, J. Pharmacol. Exp. Ther., 221 (1982) 541–546.
19 Sawada M. and Nagatsu T., Stimulation of the serotonin autoreceptor prevents the calcium-calmodulin-dependent increase of serotonin biosynthesis in rat raphe slices, J. Neurochem., 46 (1986) 963–967.
20 Sheard, M.H. and Aghajanian, G.K., Stimulation of the midbrain raphe: effect on serotonin metabolism, J. Pharmacol. Exp. Ther., 163 (1968) 425–430.
21 Sprouse, J.S. and Aghajanian, G.K., (–)-Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT_{1A} selective agonists, Eur. J. Pharmacol., 128 (1986) 295–298.
22 Starke, K., Regulation of noradrenaline release by presynaptic receptor systems, Rev. Physiol. Biochem. Pharmacol., 77 (1977) 1–124.
23 Svensson, T.H., Bunney, B.S. and Aghajanian, G.K., Inhibition of both noradrenergic and serotonergic neurons in brain by the α-adrenergic agonist clonidine, Brain Res., 92 (1975) 291–308.
24 Togashi, H., Central and peripheral effects of clonidine on the adrenal medullary function in spontaneously hypertensive rats, J. Pharmacol. Exp. Ther., 225 (1983) 191–197.
25 Westfall, T.C., Local regulation of adrenergic neurotransmission, Physiol. Rev., 57 (1977) 659–728.
26 Yamauchi, T. and Fujisawa, H., Activation of tryptophan 5-monooxygenase by calcium-dependent regulator protein, Biochem. Biophys. Res. Commun., 90 (1979) 28–35.