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Changes in the regulation of 5-hydroxytryptamine release by α_2-adrenoceptors in the rat hippocampus after long-term desipramine treatment

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Abstract

In vivo microdialysis was used to measure the effects of long-term treatment of rats with desipramine upon the regulation by α_2-adrenoceptors of serotonin (5-hydroxytryptamine, 5-HT) release from the serotonergic neurons in the hippocampus. Rats were injected with saline or desipramine, 10 mg/kg, i.p., every 12 h for 14 days. When added to the perfusion solution, brimonidine, an α_2-adrenoceptor agonist, significantly inhibited the K^+-evoked release of 5-HT in the hippocampi of saline-treated, control rats. This action of brimonidine was prevented by pretreating the rats with idazoxan, an α_2-adrenoceptor antagonist. Long-term desipramine treatment significantly reduced the inhibitory effect of brimonidine upon the K^+-evoked 5-HT release. With long-term administration of desipramine, noradrenaline content in the hippocampi was significantly decreased as compared with that of the control rats, whereas the basal noradrenaline concentration in the dialysate was significantly increased. On the other hand, both the 5-HT content of the hippocampus and the basal 5-HT concentration in the dialysate were significantly increased. The present study suggests that long-term administration of desipramine causes a functional subsensitivity of the presynaptic α_2-adrenoceptors that regulate serotonergic neuronal function in the rat hippocampus. It also supports the concept that changes in the sensitivity of α_2-adrenoceptors that regulate neurotransmitter release play an important role in the mechanism of antidepressant drug action.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Microdialysis; Desipramine; α_2-Adrenoceptor

1. Introduction

In recent years it has become apparent that presynaptic receptors have an important role in the regulation of transmitter release from neurons (Langer, 1980; Starke, 1981). A major mechanism that regulates the release of neurotransmitter at the level of the synapse appears to be feedback inhibition of release by neurotransmitter located in the synaptic cleft. For instance, noradrenaline inhibits release by acting on presynaptic α_2-adrenoceptors on noradrenergic neurons in the central nervous system. Not only autoreceptors, but also presynaptic heteroreceptors might play an important role in the regulation of neurotransmitter release in terms of interactions (cross-talk) among different neurotransmitter systems. Such a concept was proposed by Laduron (1985), and in the past decade a large number of different families of presynaptic receptors, including both auto- and heteroreceptors, have been described. In addition to the regulation of the transmitter release, transmitter biosynthesis is reported to be modulated by these presynaptic receptors (Reinhard and Roth, 1982; Yoshioka et al., 1992). Among these presynaptic heteroreceptors, a functional linkage between serotonergic and noradrenergic neurons has been reported by a number of investigators (Reinhard and Roth, 1982; Raiteri et al., 1990; Yoshioka et al., 1992; Numazawa et al., 1995). That not only highly selective noradrenaline uptake inhibitors but also highly selective

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5-HT uptake inhibitors are effective in the clinical management of depressive illness (Fawcett et al., 1989; Rudorfer and Potter, 1989; Altamura et al., 1989) is consistent with the hypothesis that the mechanism of action of noradrenaline uptake inhibitors involves an interaction with serotonergic neuronal systems in the brain at the level of presynaptic auto- and heteroreceptors. The purpose of the present study was to elucidate further such a functional relationship between noradrenergic and serotonergic neurons with respect to treatment with an antidepressant drug, desipramine.

2. Materials and methods

2.1. Animals

Male, Wistar rats (250–260 g and 8 weeks old, Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used. The animals were housed in a room with a 12-h light (07:00–19:00)-dark (19:00–07:00) cycle and were given free access to food and water. Rats were injected with a 10 mg/kg, i.p. dose of desipramine or saline every 12 h, at 8:00 and 20:00 h, for 14 days.

2.2. Brain microdialysis

On the 13th day, rats were anesthetized with ketamine (100 mg/kg, i.p.) and an 8-mm guide cannula was stereotaxically implanted into the hippocampus so that a concentric dialysis probe with a 3-mm tip (Eicom, Kyoto, Japan) could be positioned with its tip at the following co-ordinates with relation to the bregma and dural surface of the brain (Paxinos and Watson, 1986): rostral-caudal, −5.8 mm; lateral −4.8 mm; ventral −7.0 mm. The guide cannula was attached to the skull with dental cement. On the 15th day (2 days after the surgery), 12 h after the last injection of desipramine or saline, the dialysis probe was inserted into the guide cannula and perfused continuously (2 μl/min) with Ringer's solution of the following composition (mM): NaCl, 147; KCl, 4; $CaCl_2$, 2.3. In preliminary experiments it was found that the levels of 5-HT in the dialysate were high immediately after insertion of the probe but decreased to constant values within 3 h. Collection of the perfusate was started, therefore, 3 h after implantation of the probe. Nine successive 40 μl samples were collected at 20 min intervals in vials that contained 10 μl of ice-cold acetic acid (0.05 N). The samples were injected immediately into a high performance liquid chromatography (HPLC) coupled to an ECD (electrochemical detector) system.

To stimulate 5-HT release, KCl (120 mM) was administered through the perfusion system for two 10-min periods. The first administration of KCl (S_1, 0–10 min, Fig. 1) occurred at the beginning of the fourth 20-min collection period and the second administration at the beginning of the seventh (S_2, 60–70 min, Fig. 1) collection period. In control experiments, the S_2/S_1 ratio was found to be approximately 1.0 under these experimental conditions. Brimonidine was added to the perfusion solution at the time of the second administration of KCl (S_2). Idazoxan, an α_2-adrenoceptor antagonist, was injected intraperitoneally 20 min prior to the second KCl-perfusion period (S_2). In some experiments, desipramine, 10 mg/kg, i.p. was injected into normal, saline-treated rats 1 h prior to the beginning of the first KCl-perfusion period (S_1); and in other experiments desipramine, 1 or 10 μM, was added directly to the perfusate 8 min prior to the onset of the second KCl-perfusion period (S_2). At the end of the experiment, brains were rapidly removed and dissected on an ice-cold plate to isolate the hippocampus (Glowinski and Iversen, 1966; Paxinos and Watson, 1986). At the same time, brain sections were examined to determine the insertion site of the dialysis tube. The removed hippocampus was disrupted by sonication with a 5–10 times volume of 0.4 N perchloric acid containing 10 μM EDTA-2Na. Precipitated protein was removed by centrifugation at −2°C at 10000 r.p.m. for 10 min, and the 5-HT and noradrenaline content of the supernate was subsequently measured.

5-HT and noradrenaline were separated and measured by the use of a HPLC-ECD system as described previously (Matsumoto et al., 1990). The HPLC-ECD system consisted of a pump (P-500, Irika Kogyo, Kyoto, Japan) coupled to a reversed-phase column (MA-50DS, ODS, 5-μm particle size, Eicom) and an ECD (E-502, Irika Kogyo).

2.3. Drugs

The following compounds were used: 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Co., St. Louis, MO, USA), desipramine hydrochloride (Sigma), brimonidine (Reckitt and Colman, Hull, UK), idazoxan hydrochloride (Reckitt and Colman), and ketamine hydrochloride (Sankyo Co., Tokyo, Japan).

2.4. Calculations and statistical analysis

The 5-HT in the dialysates is expressed as a percentage of the absolute amount of 5-HT in the dialysate collected during the third 20-min collection period, i.e. the period immediately before KCl was added to the perfusate. The amount of 5-HT released during K^+ stimulation is expressed either as the amount in the dialysate collected during the fourth collection period (S_1) less the average of the amounts collected during the third and fifth collection periods, or as the amount collected during the seventh collection period (S_2) less

the average of the amounts collected during the sixth and eighth collection periods.

All results are given as means ± S.E.M. Statistical comparisons were carried out using analysis of variance followed by Student's *t*-test. Values of *P* less than 5% were considered significant.

3. Results

3.1. Effect of K^+ on 5-HT release and its blockade by brimonidine

When introduced into the perfusion buffer, KCl (120 mM) increased the concentration of 5-HT in the dialysate (Fig. 1). The mean basal output of 5-HT from the serotonergic neurons in the rat hippocampus was 2.3 ± 0.4 pg/40 μl of dialysate. KCl was introduced into the perfusion medium for 10 min periods at the beginning of the fourth (S_1) and seventh (S_2) 20-min collection periods (Fig. 1). The mean S_2/S_1 ratio in these experiments was 1.02 ± 0.09 (Fig. 2, $n = 8$). Brimonidine, an agonist selective for α_2-adrenoceptors, inhibited the K^+-induced release of 5-HT when added to the perfusion medium in a concentration of 10 μM simultaneously with the second stimulation with KCl (S_2) (Fig. 1). Brimonidine, when administered without the concomitant administration of KCl, did not alter the spontaneous release of 5-HT.

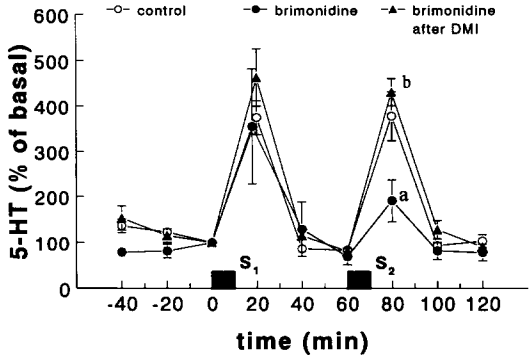

Fig. 1. Inhibition by brimonidine of hippocampal 5-HT release elicited by 120 mM KCl, and the effect of long-term desipramine administration. Ordinate: amount of 5-HT in the dialysate expressed as a percent of the amount in the third collection period, the period immediately before the first administration of KCl. Abscissa: time beginning with the first and ending with the ninth collection period. Dialysates were collected starting 180 min after insertion of the dialysis probe. S_1 and S_2, 10 min periods during which the concentration of KCl was increased in the Ringer's perfusion buffer. Open circles, control experiments; solid circles, brimonidine, 10 μM, co-perfused with the second administration of KCl (S_2); solid triangles, rats treated with long-term desipramine (DMI), 10 mg/kg, i.p, twice daily. Each point represents the mean of 6-8 determinations. [a]Indicates a significant difference from S_1. [b]Indicates a significant difference from brimonidine-control group (solid circles). Vertical bars represent the S.E.M.

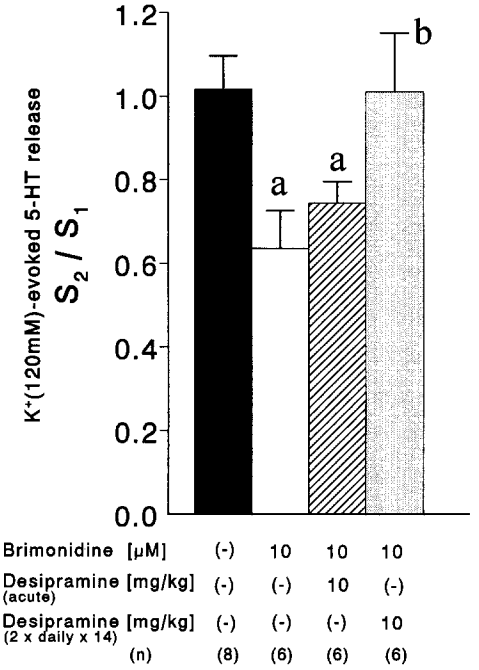

Fig. 2. Effects of brimonidine on K^+-evoked 5-HT release, antagonism by idazoxan and effects of long-term desipramine. Ordinate: S_2/S_1 ratios. Abscissa: concentrations of brimonidine and desipramine. [a]Indicates a significant difference from control group (solid column). [b]Indicates a significant difference from brimonidine-control group (open column). Vertical bars represent the S.E.M.

3.2. Antagonism by idazoxan of the brimonidine-induced inhibition of 5-HT release

To determine whether the inhibitory actions of brimonidine on 5-HT release were mediated by α_2-adrenoceptors, the actions of brimonidine were studied in rats treated with idazoxan, a selective α_2-adrenoceptor antagonist, at a dose of 5 mg/kg i.p. Pretreatment with idazoxan prevented the inhibition of 5-HT release by 10 μM brimonidine. The mean S_2/S_1 ratio was 0.87 ± 0.12 ($n = 6$; $P < 0.05$). These observations are consistent with the hypothesis that brimonidine inhibits hippocampal 5-HT release by acting at the α_2-adrenoceptors.

3.3. Effects of desipramine on 5-HT release

Long-term administration of desipramine resulted in significant decreases in the inhibition of 5-HT release by brimonidine. Administration of desipramine hydrochloride, 10 mg/kg, twice daily for 2 weeks prevented the inhibition of 5-HT release by brimonidine, 10 μM (Fig. 1). The mean S_2/S_1 ratio was 1.01 ± 0.14 (Fig. 2, $n = 6$). When rats were injected with desipramine, 10 mg/kg, i.p., 1 h prior to S_1, the inhibitory action of brimonidine, 10 μM, was not altered significantly, and the S_2/S_1 ratio was 0.74 ± 0.10 ($n = 6$). Furthermore,

Table 1
Effects of long-term treatment with desipramine on the monoamine content of the hippocampus and on basal concentrations in the dialysate. Each value is expressed as mean ± S.E.M.

	Hippocampal content (ng/g wet weight)		Concentration in dialysate (pg/sample)	
	Noradrenaline	5-HT	Noradrenaline	5-HT
Control ($n = 8$)	268.0 ± 35.4	288.9 ± 63.9	2.4 ± 0.2	2.3 ± 0.4
Desipramine ($n = 6$)	139.5 ± 15.3 [a]	542.7 ± 39.7 [a]	16.4 ± 2.9 [a]	5.5 ± 0.9 [a]

[a] Indicates a significant difference ($P < 0.01$) between control values.

when desipramine, 1 and 10 μM, was added to the perfusate 8 min prior to the onset of S_2, the effects of brimonidine were only slightly attenuated. S_2/S_1 ratios were 0.79 ± 0.08 ($n = 4$) at the 1-μM concentration and 0.81 ± 0.10 ($n = 3$) at the 10-μM concentration of desipramine.

3.4. Effect of long-term administration of desipramine on 5-HT and noradrenaline content

The 5-HT content in the hippocampus, as well as basal 5-HT concentration in the dialysate, were significantly increased by long-term treatment with desipramine (Table 1). While noradrenaline content in the hippocampus was significantly decreased by long-term treatment with desipramine, basal noradrenaline concentration in the dialysate was significantly increased (Table 1).

4. Discussion

The present study demonstrates that brimonidine, a selective α_2-adrenoceptor agonist (Cambridge, 1981), inhibits the depolarization-induced release of endogenous 5-HT in the hippocampus of freely moving rats and that this inhibition is prevented by pretreatment with idazoxan, a selective α_2-adrenoceptor antagonist. The release in vitro of 5-HT from different rat brain preparations is inhibited by the activation of α_2-adrenoceptors located on serotonergic nerve terminals (Starke and Montel, 1973; Frankhuyzen and Mulder, 1980; Göthert and Huth, 1980; Maura et al., 1982). The present findings in vivo are consistent with those of previous studies that used in vitro preparations and also support the hypothesis that noradrenergic neurons, via stimulation of α_2-adrenoceptors on serotonergic nerve terminals, modulate the release of 5-HT in the rat brain.

In the present study, long-term administration of desipramine significantly decreased the sensitivity of α_2-adrenoceptors on serotonergic nerve terminals in the rat hippocampus. This functional decrease in the sensitivity of α_2-adrenoceptors requires long-term administration of desipramine since neither acute treatment with desipramine nor addition of desipramine directly to the perfusate during depolarization-induced release of endogenous 5-HT significantly modified α_2-adrenoceptor function. Numerous studies have reported that tricyclic antidepressants, when administered chronically, decrease the number and/or the sensitivity of α_2-adrenoceptors on noradrenergic neurons within and outside of the central nervous system (Crews and Smith, 1978, 1980; Smith and Hollingsworth, 1984). One explanation of the present findings is that desipramine, an antidepressant drug that is a relatively selective inhibitor of noradrenaline uptake by noradrenergic neurons, influences serotonergic neuronal function via the α_2-adrenergic heteroreceptors located at serotonergic nerve terminals. Morphological studies of the organization of the monoamine systems and psychopharmacological studies have long suggested a functional linkage between noradrenergic and serotonergic neuronal systems in the brain. Previous studies of the effects of antidepressants on the β-adrenoceptor-coupled adenylate cyclase system in rat brain have provided support for this aminergic link. Thus, an intact serotonergic neuronal input is required for the down-regulation of cortical β-adrenoceptors by tricyclic antidepressants (Janowsky et al., 1982; Drumbrille-Ross and Tang, 1983; Manier et al., 1987). A link between the two aminergic receptor systems in in vitro studies was also noted above. In hippocampal slices, electrical stimulation-induced release of [^{3}H]5-HT is inhibited by α_2-adrenoceptor stimulation (Frankhuyzen and Mulder, 1980). Furthermore, clinical evidence that not only selective noradrenaline reuptake inhibitors but also selective 5-HT reuptake inhibitors are effective in treating depressive illness (Fawcett et al., 1989; Rudorfer and Potter, 1989; Altamura et al., 1989) are consistent with this hypothesis. Thus, the present findings with long-term desipramine administration further support the hypothesis that a functional linkage exists between serotonergic and noradrenergic systems in the rat hippocampal formation in vivo.

The present experiments also show that long-term administration of desipramine decreases the noradrenaline content of the hippocampus. This finding, however, is at variance with reports of no change in the noradrenaline content of the hypothalamus and the

caudate nucleus after chronic antidepressant administration (Karoum et al., 1984) and a slight reduction of noradrenaline content in the whole brain after 3 weeks of impramine administration (Schildkraut et al., 1970). The differences between our findings and those of other investigators might be attributed to differences in the brain regions analyzed and differences in drug doses and the duration of drug administration. Chronic desipramine administration was reported to decrease tyrosine hydroxylase activity (Segal et al., 1974; Nestler et al., 1990) and to reduce neuronal firing rate in the locus coeruleus (Svensson and Usdin, 1978). It is conceivable that reduction of noradrenaline content induced by desipramine might be due to a decrease in noradrenaline synthesis. In contrast, hippocampal 5-HT content was increased by long-term administration of desipramine. When administered chronically, a number of antidepressant drugs, including desipramine, have been reported to reduce the turnover of 5-HT in rat (Fuxe et al., 1982) and to reduce the concentration of 5-HIAA in human cerebrospinal fluid (Potter et al., 1981). On the other hand, with respect to the increased 5-HT content, we reported previously that α_2-adrenoceptors modulate 5-HT synthesis in the rat hippocampus (Yoshioka et al., 1992). In this context, the increase in 5-HT content induced by desipramine might be due to a disinhibition of this modulatory system; namely, an effect of down-regulating α_2-adrenoceptors on serotonergic nerve terminals.

With respect to basal noradrenaline levels in the dialysate, long-term desipramine administration produced a marked increase in noradrenaline concentration as compared with the control rats. It is conceivable that this effect was due to an intrinsic property of desipramine, i.e., noradrenaline reuptake inhibition in spite of the reduction of noradrenaline synthesis. In a preliminary study, acute administration of desipramine (10 mg/kg, i.p.) produced an increase in noradrenaline concentration in the dialysate but did not affect the concentration of 5-HT in the dialysate. However, the basal 5-HT concentration in the dialysate was also increased by long-term desipramine administration. One explanation for this finding is that during long-term desipramine treatment both α_2-autoreceptor and α_2-heteroreceptors are down regulated. Although the former means less autoinhibition of release and therefore more noradrenaline in the extracellular fluid, the latter may mean less inhibition by noradrenaline of 5-HT release and therefore more 5-HT in the extracellular fluid. Another explanation for the increase in the 5-HT concentration in the dialysate in the present study is that it results from increases in spontaneous release secondary to stimulation of 5-HT synthesis and not from a blockade of reuptake by desipramine.

In conclusion, there is an α_2-adrenoceptor-mediated functional modulation of 5-HT release from the serotonergic neurons in the rat hippocampus in vivo. In addition, long-term treatment with desipramine produces a decrease in the sensitivity of the α_2-adrenoceptors that modulate the neuronal release of 5-HT.

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