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Evidence That Exogenous but Not Endogenous Norepinephrine Activates the Presynaptic *Alpha*-2 Adrenoceptors on Serotonergic Nerve Endings in the Rat Hypothalamus[1]

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ABSTRACT

In superfused rat hypothalamic slices, clonidine, norepinephrine (NE) and 6-fluoronorepinephrine reduced the electrically evoked release of recently taken up [^{3}H]-5-hydroxytryptamine (5-HT). The inhibitory action of these drugs involves the activation of presynaptic *alpha*-2 adrenoceptors and it was antagonized by the *alpha* adrenoceptor antagonists phentolamine or RX 781094. In contrast to the facilitating effect of *alpha*-2 adrenoceptor antagonists on the electrically evoked [^{3}H]NE overflow in rabbit hypothalamic slices, neither phentolamine nor RX 781094 modified the stimulation-evoked release of [^{3}H]-5-HT at concentrations which completely antagonized the inhibitory action of NE, 6-fluoronorepinephrine and clonidine on 5-HT neurotransmission. In the presence of cocaine, which inhibits the neuronal uptake of NE and increases the concentration of this neurotransmitter in the synaptic gap, *alpha*-2 adrenoceptor antagonists were still unable to modify the electrically evoked release of [^{3}H]-5-HT. It is concluded that presynaptic *alpha*-2 adrenoceptors present on serotonergic nerve endings in the hypothalamus are not activated by endogenous NE and do not seem to play a physiological role in the regulation of serotonergic neurotransmission. However, presynaptic inhibitory *alpha*-2 adrenoceptors can be acted upon by exogenous agonists to inhibit 5-HT release. On the other hand, the presynaptic *alpha*-2 adrenoceptors on noradrenergic nerve terminals in the rabbit and rat hypothalamus are acted upon by released NE because the *alpha*-2 adrenoceptor antagonists by themselves increase the electrically evoked release of [^{3}H]NE.

The presynaptic inhibition of peripheral and central noradrenergic neurotransmission by *alpha*-2 adrenoceptor agonists is by now well established (for reviews see Langer, 1974, 1977, 1980; Starke, 1977). These presynaptic *alpha*-2 adrenoceptors on noradrenergic nerves are involved in a negative feedback mechanism which modulates the release of NE during nerve stimulation (Langer, 1980). In central serotonergic neurons a presynaptic inhibitory 5-HT autoreceptor modulates serotonin release through a negative feed-back mechanism whereby the neurotransmitter can modulate its own release (Cerrito and Raiteri, 1979; Göthert and Weinheimer, 1979; Langer and Moret, 1982). In addition, the presence of presynaptic inhibitory *alpha* adrenoceptors on serotonergic nerve terminals was reported in the rat brain cortex (Göthert and Huth, 1980), hippocampus (Frankhuyzen and Mulder, 1980) and hypothalamus (Galzin *et al.*, 1982b). These receptors were subsequently characterized as being of the *alpha*-2 subtype (Göthert *et al.*, 1981). A physiological involvement of these presynaptic inhibitory *alpha*-2 adrenoceptors in the modulation of 5-HT neurotransmission was proposed by Göthert and Huth (1980) because phentolamine enhanced the stimulation-evoked overflow of [^{3}H]-5-HT from rat cortex slices. However, in a more recent article it was reported that the *alpha*-2 adrenoceptor antagonists yohimbine and rauwolscine unexpectedly decreased the stimulation-evoked overflow of [^{3}H]-5-HT from rat cortex slices (Göthert *et al.*, 1981). In order to clarify the possible physiological role of *alpha*-2 adrenoceptors on central serotonergic neurotransmission, we carried out our study in perfused rat hypothalamic slices. The effects of *alpha*-2 adrenoceptor agonists and antagonists were also determined on the electrically evoked release of [^{3}H]NE in rabbit hypothalamic slices.

Materials and Methods

Rabbit hypothalamic slices. The methods employed were essentially the same as described by Galzin *et al.* (1982a). Briefly, male rabbits (1.5–3 kg) were killed by decapitation and the brains were quickly removed. Hypothalami were dissected and chopped at a 0.4 mm thickness by a McIlwain tissue chopper. The slices were then immersed in cold Krebs' solution of the following millimolar composition: NaCl, 118; KCl, 4.7; glucose, 11.1; $NaHCO_3$, 25.0; $MgCl_2$, 1.2; NaH_2PO_4, 1.0; $CaCl_2$, 1.3; ascorbic acid, 0.11; and disodium EDTA, 0.004. The endog-

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[1] Some of these results were presented at the Meeting of the British Pharmacological Society (Galzin *et al.*, 1982b).
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ABBREVIATIONS: NE, norepinephrine; 5-HT, 5-hydroxytryptamine; 6F-NE, 6-fluoronorepinephrine.

enous stores of NE were labeled by incubating the slices with 0.33 μM (±)-[7-^{3}H]NE (specific activity, 13.274 Ci/mmol; New England Nuclear, Boston, MA) during 15 min in Krebs' solution bubbled with 95% O_2-5% CO_2 and maintained at 37°C. After a 2-min wash in 25 ml of Krebs' solution, slices were transferred to glass superfusion chambers and superfused at 0.5 ml/min with Krebs' solution at 37°C during 60 min before the first period of electrical stimulation was carried out. The superfusate was collected in 4-min samples. [^{3}H]NE release was elicited by a 2-min period of electrical stimulation (5 Hz, 26 mA, 2 msec duration) delivered by a Grass stimulator model S44. Two periods of electrical stimulation were applied in each experiment (S_1 and S_2) at 60 and 104 min after the end of the incubation with [^{3}H]NE, respectively. At the end of the experiment, the slices were solubilized in Soluene and the tritium content of the slices and superfusate samples was determined by liquid scintillation counting. The overflow of labeled transmitter elicited by electrical stimulation was expressed as fractional release of the total radioactivity present in the tissue at the onset of stimulation (Pelayo *et al.*, 1980).

Rat hypothalamic slices. Male Sprague-Dawley rats weighing 180 to 200 g were decapitated and their brains immediately removed and dissected. Slices of 0.4 mm thickness from the hypothalamus were incubated for 30 min at 37°C in Krebs' solution containing 0.1 μM [^{3}H]-5-HT creatinine sulfate (Amersham, Des Plains, IL; specific activity, 12 Ci/mmol) and bubbled with a mixture of 95% O_2-5% CO_2. The composition of the Krebs' solution was the same as described above. At the end of the incubation period, slices were transferred to glass superfusion chambers and superfused at 0.5 ml/min with Krebs' solution prewarmed to 37°C 60 min before the first period of stimulation was carried out.

[^{3}H]-5-HT release was elicited by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec duration). The rest of the protocol was essentially the same as that described in the previous section.

In some experiments, rat hypothalamic slices were labeled with [^{3}H]NE (0.33 μM) for 15 min and superfused under the same conditions as described above. [^{3}H]NE release was elicited by a 2-min period of electrical stimulation at 3 Hz (20 mA, 2 msec duration). The rest of the protocol was the same as that employed with rabbit hypothalamic slices.

Results are expressed as mean ± S.E.M. Unpaired Student's *t* test and the Mann-Whitney two-tail test were used for statistical comparisons.

Labeling studies. In order to test the selectivity of labeling transmitter stores with [^{3}H]NE in noradrenergic neurons and [^{3}H]-5-HT in serotonergic neurons, labeling studies were performed in rat hypothalamic slices. After a preincubation period of 15 min at 37°C with Krebs' solution or with Krebs' solution in the presence of the NE uptake inhibitors cocaine (1 μM) or desmethylimipramine (0.3 μM) or the 5-HT uptake inhibitor citalopram (1 μM), the endogenous stores of neurotransmitter were labeled by incubation at 37°C for 30 min with either 0.1 μM [^{3}H]-5-HT creatinine sulfate (the same as above) or 0.1 μM (±)-[^{3}H]NE (New England Nuclear; specific activity, 5.8 Ci/mmol). During the preincubation and incubation periods, the Krebs' solution was continuously bubbled with a mixture of 5% CO_2 in O_2. At the end of the incubation period, each slice was set up in a special chamber and superfused with Krebs' solution under the same conditions as described above. After 52 min of superfusion, the slices were solubilized and the radioactivity in the slices was measured as indicated for rabbit hypothalamic slices.

Drugs. The following drugs were used: clonidine hydrochloride (Boehringer Mannheim Corp., New York, NY); *l*-NE bitartrate (Sigma Chemical Co., St. Louis, MO); 6F-NE hydrochloride and yohimbine hydrochloride (Sigma); phentolamine methanesulfonate (Ciba-Geigy Corp., Summit, NJ); RX 781094 {2-[2-(1,4-benzodioxanyl)]-2-imidazolidine hydrochloride} and cocaine hydrochloride (La Coopération Pharmaceutique Française); prazosin hydrochloride (Pfizer Inc., New York, NY); phenylephrine hydrochloride (Koch-Light); citalopram (Lu 10171 {1-[3-(dimethylamino)propyl)-1-(*p*-fluorophenyl)-5-phthalanecarbonitrile} (Lundbeck, Copenhagen, Denmark). 6F-NE and RX 781094 were synthetized by the Department of Chemistry, Synthelabo, Paris, France.

Results

Antagonism by yohimbine or RX 781094 of the inhibitory effect of NE on the electrically evoked overflow of [^{3}H]NE in rabbit hypothalamic slices. In the controls, the fraction of the total tissue radioactivity released by electrical stimulation at 5 Hz was approximately 1% of the tissue stores (table 1). The electrically evoked release of [^{3}H]NE from rabbit hypothalamic slices was shown to be entirely calcium-dependent (Galzin *et al.*, 1982a).

Exposure to the neuronal uptake inhibitor cocaine (10 μM) increased by itself the overflow of [^{3}H]NE without affecting the spontaneous outflow of radioactivity (table 1). When cocaine was added to the medium 40 min before S_1 and kept throughout the experiment, the ratio S_2/S_1 did not differ from unity (table 1). Under these experimental conditions, it was previously demonstrated that exogenous NE inhibited in a concentration-dependent manner the overflow of ^{3}H-transmitter elicited by electrical stimulation with a maximal inhibitory effect at 0.1 μM (Galzin *et al.*, 1982a; fig. 1).

When the *alpha*-2 adrenoceptor antagonist yohimbine (0.1 μM) was added along with cocaine (10 μM) 40 min before S_1 and kept in the medium for the rest of the experiment, there was a 3-fold increase in the absolute value of fractional release of [^{3}H]NE at 5 Hz, whereas the ratio S_2/S_1 did not differ from unity (table 1). Under these experimental conditions, the inhibitory effects of 0.1 and 1 μM NE on [^{3}H]NE overflow were significantly antagonized by yohimbine when compared with the corresponding controls (fig. 1).

The selective *alpha*-2 adrenoceptor antagonist RX 781094 (Chapleo *et al.*, 1981; Langer and Pimoule, 1982; Langer *et al.*, 1983) at 1 μM, when added together with cocaine (10 μM) 40 min before S_1, increased by nearly 5-fold the electrically evoked release of [^{3}H]NE in S_1. Under these conditions, the ratio S_2/S_1 was not different from unity (table 1). The inhibitory effect of 0.1 and 1 μM NE on [^{3}H]NE overflow was clearly antagonized by this concentration of RX 781094 as shown in figure 1.

Effects of *alpha*-2 adrenoceptor antagonists on the electrically evoked release of [^{3}H]NE in rabbit or rat hypothalamic slices. When the *alpha*-2 adrenoceptor antagonist yohimbine was added to the superfusion medium 20 min before S_2, it increased in a concentration-dependent manner the stimulation-evoked release of [^{3}H]NE without affecting the spontaneous outflow of radioactivity (fig. 2). A maximal increase of 300% was obtained with 1 μM yohimbine and the concentration that elicited 50% of this maximal effect (EC_{50}), calculated by computer analysis, was 0.085 μM.

When RX 781094 was added to the superfusion medium 20 min before S_2, it also increased the overflow of [^{3}H]NE (fig. 3), but the concentration-effect curve was not of a classical sigmoid shape (fig. 3). These results suggested that RX 781094 might be acting on more than one site to increase [^{3}H]NE overflow. Therefore, similar experiments were carried out in the presence of cocaine (10 μM) to inhibit neuronal uptake. In the presence of cocaine, RX 781094 enhanced the stimulation-evoked release of [^{3}H]NE in a concentration-dependent manner, with a maximal increase of 350% at a concentration of 30 μM (fig. 3). The EC_{50} for RX 781094, in the presence of cocaine, was 2.4 μM. These results suggest that at high concentrations RX 781094

TABLE 1

Electrically evoked overflow of [^{3}H]NE from rabbit hypothalamic slices: effects of cocaine and *alpha*-2 adrenoceptor antagonists

The radioactivity retained by the tissue after 130 min of superfusion was 58.4 ± 5.0 nCi/slice (n = 7) in control experiments. The radioactivity retained by tissues in all other experimental groups was not significantly different from this control value. Drugs in the concentrations indicated were added to the medium 40 min before S_1 and remained present throughout the rest of the experiment. Shown are mean values ± S.E.M. n, number of experiments.

Experimental Group	n	^{3}H-Transmitter Overflow[a] (Fractional release × 10^{-2})			Spontaneous Outflow (Fractional release × 10^{-2})		
		S_1	S_2	Ratio[b] S_2/S_1	Sp_1	Sp_2	Ratio[c] Sp_2/Sp_1
Control	7	1.03 ± 0.11	1.01 ± 0.10	0.99 ± 0.06	1.05 ± 0.03	0.79 ± 0.04	0.75 ± 0.02
Cocaine, 10 μM (S_1–S_2)	7	1.85 ± 0.29*	1.72 ± 0.28*	0.95 ± 0.04	1.01 ± 0.04	0.78 ± 0.03	0.77 ± 0.01
Cocaine, 10 μM + yohimbine, 0.1 μM (S_1–S_2)	5	3.07 ± 0.46**	2.99 ± 0.41**	0.98 ± 0.05	1.03 ± 0.07	0.79 ± 0.06	0.76 ± 0.01
Cocaine, 10 μM + RX 781094, 1 μM (S_1–S_2)	5	4.87 ± 1.06**	4.30 ± 0.79**	0.93 ± 0.05	1.01 ± 0.01	0.82 ± 0.03	0.81 ± 0.02

[a] Fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (5 Hz, 2 msec, 26 mA) 60 min (S_1) and 104 min (S_2) after the end of the incubation with [^{3}H]NE.

[b] Ratio of fractional release obtained between S_2 and S_1.

[c] Ratio between the spontaneous outflow of radioactivity obtained during the 4 min preceding the second stimulation (Sp_2) and the corresponding fraction of radioactivity released spontaneously before the first stimulation period (Sp_1).

* $P < .05$; ** $P < .01$ when compared with the corresponding control value.

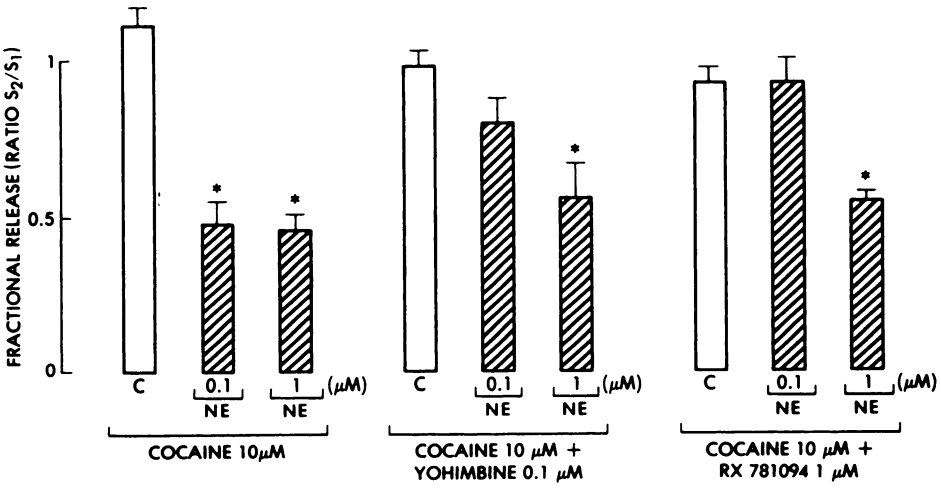

Fig. 1. Effect of yohimbine or RX 781094 on the inhibition by NE of the stimulation evoked release of [^{3}H]NE from rabbit hypothalamic slices. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (5 Hz, 2 msec, 26 mA) expressed as the ratio obtained between S_2 and S_1 carried out within the same experiment. NE (0.1 or 1 μM) was added to the medium 20 min before S_2. Cocaine (10 μM) was added to the superfusion medium 40 min before S_1 and remained present throughout the experiment. When used, yohimbine (0.1 μM) or RX 781094 (1 μM) was added to the medium 40 min before S_1 and remained present throughout the experiment. The mean values ± S.E.M. of at least three experiments per group are shown. The fractional release of radioactivity obtained in S_1 was 1.89 ± 0.22% of tissue stores (n = 5) in control (C) experiments; 3.07 ± 0.46% (n = 5) in the presence of yohimbine (0.1 μM); and 4.87 ± 1.06% (n = 5) in the presence of RX 781094 (1 μM). * $P < .01$ when compared with the corresponding control.

can inhibit the neuronal uptake of NE and this action contributes to the increase in [^{3}H]NE overflow once the facilitation in release due to blockade of presynaptic *alpha*-2 adrenoceptors is already at the maximum (fig. 3).

In rat hypothalamic slices prelabeled with [^{3}H]NE, the fraction of the total radioactivity released by electrical stimulation at 3 Hz was 1.01 ± 0.13% (n = 8). The control ratio S_2/S_1 was 0.90 ± 0.11 (n = 8). Under these experimental conditions, the *alpha*-2 adrenoceptor antagonists yohimbine (1 μM) or RX 781094 (1 μM), when added 20 min before S_2, significantly increased the stimulation-evoked release of [^{3}H]NE without affecting the spontaneous outflow of radioactivity (S_2/S_1 = 2.00 ± 0.14, n = 4 for yohimbine, 1 μM, and S_2/S_1 = 1.74 ± 0.20, n = 4 for RX 781094, 1 μM; $P < .01$ when compared with the control value).

Effects of cocaine, desipramine and citalopram on the accumulation of [^{3}H]-5-HT or [^{3}H]NE in rat hypothalamic slices. In experiments designed to study the uptake and retention of [^{3}H]-5-HT in slices of the hypothalamus, exposure to 1 μM cocaine or 0.3 μM desipramine did not modify the accumulation of [^{3}H]-5-HT when compared with the controls (table 2). On the other hand, exposure to citalopram (1 μM) decreased significantly the labeling of the slices of the rat hypothalamus with [^{3}H]-5-HT (table 2). In the presence of 1 μM citalopram the accumulation of [^{3}H]-5-HT was inhibited by approximately 80%. In separate experiments in which the accumulation of [^{3}H]NE was studied, exposure to 1 μM citalopram had no effect whereas cocaine (1 μM) or desipramine (0.3 μM) decreased by 63 and 55%, respectively, the labeling of the slices of the rat hypothalamus with [^{3}H]NE (table 2).

Antagonism by phentolamine or RX 781094 of the inhibitory effects of *alpha* adrenoceptor agonists on the electrically evoked release of [^{3}H]-5-HT from rat hypothalamic slices. The effect of clonidine on the release of [^{3}H]-5-HT elicited by electrical stimulation is shown in figure 4. Clonidine (0.001–1 μM) decreased the electrically evoked release of [^{3}H]-5-HT in a concentration-dependent manner. These concentrations of clonidine did not affect the spontaneous outflow of radioactivity (table 3). Phentolamine at 0.1 μM, a concentration which did not modify by itself the electrically evoked release of [^{3}H]-5-HT (table 5), competitively antagonized the inhibitory effect of the *alpha*-2 adrenoceptor agonist and resulted in a parallel shift to the right of the dose-response curve to clonidine (fig. 4). Exposure to NE (0.03–0.3

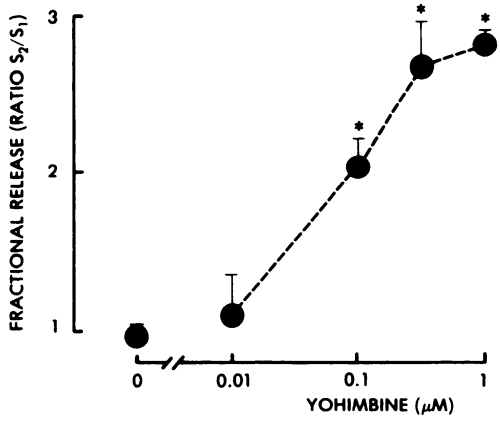

Fig. 2. Effect of yohimbine on the stimulation-evoked release of [^{3}H]NE from rabbit hypothalamic slices. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (5 Hz, 2 msec, 26 mA) expressed as the ratio obtained between S_2 and S_1 carried out within the same experiment. *Abscissa*: concentration of yohimbine (micromolar). Yohimbine was added to the superfusion medium 20 min before S_2. Each point represents the mean ± S.E.M. (vertical bars) of at least three experiments per group. The fractional release of radioactivity obtained in S_1 was 1.03 ± 0.11% of tissue stores (n = 7). * P < .01 when compared with the control value.

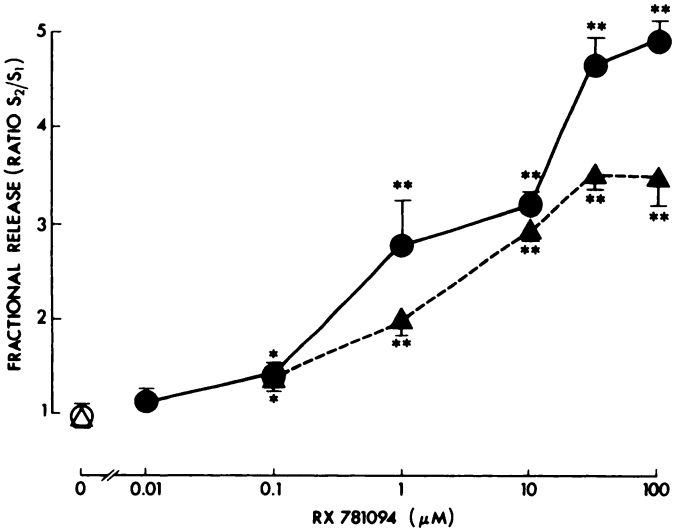

Fig. 3. Effects of RX 781094 in the presence or in the absence of cocaine on the electrically evoked release of [^{3}H]NE from rabbit hypothalamic slices. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (5 Hz, 2 msec, 26 mA) expressed as the ratio obtained between S_2 and S_1 carried out within the same experiment. *Abscissa*: concentrations of RX 781094 (micromolar). ●, RX 781094; ▲, RX 781094 + cocaine (10 μM). RX 781094 was added to the superfusion medium 20 min before S_2. When used, cocaine (10 μM) was added to the medium 40 min before S_1 and maintained throughout the rest of the experiment. Each point represents the mean ± S.E.M. (vertical bars) of at least four experiments per group. The fractional release of radioactivity obtained in S_1 was 1.03 ± 0.11% of tissue stores (n = 7) in control experiments and 1.85 ± 0.29% (n = 7) in the presence of cocaine (10 μM). * P < .05; ** P < .01 when compared with the corresponding control value.

TABLE 2

Effect of inhibitors of neuronal uptake on the accumulation of [^{3}H]-5-HT or [^{3}H]NE in rat hypothalamic slices

The values represent [^{3}H]-5-HT or [^{3}H]NE accumulation in rat hypothalamic slices expressed in femtomoles per slice and in percentage of controls, determined after a 30-min incubation with 0.1 μM [^{3}H]-5-HT (specific activity, 10–20 Ci/mmol) or 0.1 μM [^{3}H]NE (specific activity, 5.8 Ci/mmol) at 37°C. Drugs in the concentration indicated were present in the incubation medium 15 min before the addition of the radioactive transmitter and throughout the 30-min period of incubation. Shown are mean values ± S.E.M. n, number of experiments; DMI, desmethylimipramine.

Experimental Group	[^{3}H]-5-HT			[^{3}H]NE		
	n	fmol/slice	%	n	fmol/slice	%
Control	6	3757 ± 237	100	10	5133 ± 185	100
Citalopram, 1 μM	5	723 ± 19*	19	5	6094 ± 530	118
Cocaine, 1 μM	6	3669 ± 90	98	6	1911 ± 103**	37
DMI, 0.3 μM	5	3682 ± 131	98	4	2343 ± 171*	45

* P < .05; ** P < .001 when compared with the control value.

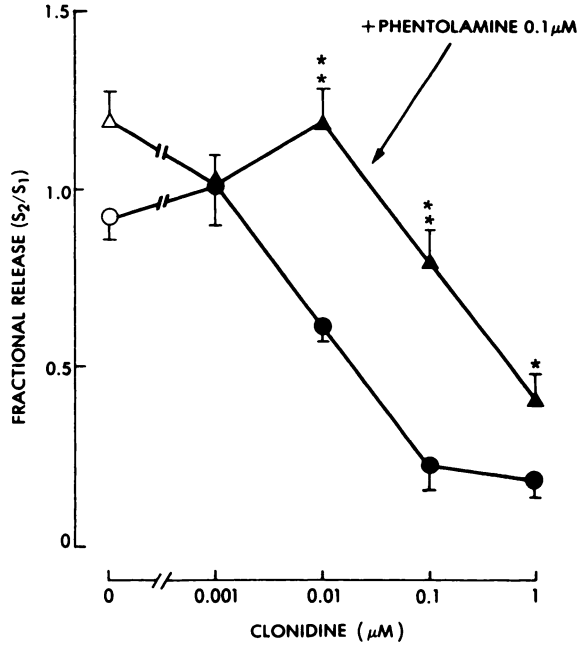

Fig. 4. Effect of phentolamine on the inhibition by clonidine of the release of [^{3}H]-5-HT elicited by electrical stimulation from slices of the rat hypothalamus. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio (S_2/S_1) obtained between S_2 and S_1 carried out within the same experiment. ○, control; ●, clonidine; △, control in the presence of phentolamine (0.1 μM) during S_1 and S_2; ▲, clonidine added before S_2 in the presence of phentolamine (0.1 μM) throughout the experiment. *Abscissa*: concentrations of clonidine (micromolar). Clonidine in the concentrations indicated was added to the medium 20 min before S_2. Phentolamine (0.1 μM) was added to the medium 20 min before S_1 and maintained for the rest of the experiment. Each point represents mean values ± S.E.M. of at least six experiments per group. * P < .05; ** P < .005 when compared with the corresponding values obtained in the absence of phentolamine. Clonidine, when present alone, significantly decreased [^{3}H]-5-HT release at 0.01 μM (P < .005), 0.1 μM (P < .001) and 1 μM (P < .005), when compared with the control value in the absence of drugs. The values of fractional release during the first period of electrical stimulation (S_1) were control, 1.92 ± 0.08% (n = 62); and phentolamine (0.1 μM), 1.88 ± 0.06% (n = 68).

μM) or 6F-NE (0.03–0.3 μM) before S_2 decreased the release of [^{3}H]-5-HT elicited by electrical stimulation in a concentration-dependent manner (fig. 5, A and B, respectively). The concentrations of the two catecholamines employed in these experiments did not modify the basal efflux of radioactivity in spite of the fact that the neuronal uptake of monoamines was not inhibited (table 3). The inhibitory effects of NE and 6F-NE on the electrically evoked release of [^{3}H]-5-HT were also antagonized by phentolamine (fig. 5, A and B). When the selective *alpha*-2 adrenoceptor antagonist RX 781094 (0.1 μM) was added to the medium 20 min before S_1 and maintained throughout the experiment, the ratio S_2/S_1 was 0.87 ± 0.06 (n = 6).

TABLE 3

Effect of *alpha* adrenoceptor agonists and antagonists on the spontaneous outflow of radioactivity from rat hypothalamic slices prelabeled with [^{3}H]-5-HT

Drugs in the concentrations indicated were added to the medium 20 min before S_2. Shown are mean values ± S.E.M. *n*, number of experiments.

Experimental Group	*n*	Spontaneous Outflow[a] (Fractional release × 10^{-2})		Ratio[b] Sp_2/Sp_1	Tissue Content[c]
		Sp_1	Sp_2		
					fmol/slice
Control	8	2.22 ± 0.09	1.75 ± 0.11	0.78 ± 0.03	2244 ± 120
Clonidine					
0.001 μM	8	2.21 ± 0.08	1.63 ± 0.07	0.74 ± 0.02	2448 ± 146
0.01 μM	6	2.45 ± 0.07	1.85 ± 0.06	0.75 ± 0.01	2076 ± 87
0.1 μM	8	2.24 ± 0.12	1.73 ± 0.08	0.78 ± 0.02	2241 ± 78
1 μM	6	2.20 ± 0.07	1.67 ± 0.06	0.76 ± 0.02	2253 ± 105
Norepinephrine					
0.03 μM	6	2.21 ± 0.08	1.72 ± 0.05	0.78 ± 0.01	2620 ± 219
0.3 μM	8	2.09 ± 0.03	1.61 ± 0.03	0.77 ± 0.01	2576 ± 143
6-F-NE					
0.03 μM	6	1.97 ± 0.11	1.52 ± 0.08	0.77 ± 0.01	2791 ± 136
0.3 μM	6	2.29 ± 0.07	1.72 ± 0.06	0.75 ± 0.01	2565 ± 173
Phentolamine					
0.1 μM	5	2.34 ± 0.08	1.80 ± 0.06	0.77 ± 0.02	2218 ± 143
1 μM	9	2.30 ± 0.13	1.68 ± 0.07	0.74 ± 0.03	2540 ± 281

[a] Spontaneous outflow of tritium expressed as the fraction of total tissue radioactivity released in the 4-min sample preceding the first period of stimulation (Sp_1) or the second period of stimulation (Sp_2).

[b] Ratio between the spontaneous outflow of radioactivity obtained during the 4 min preceding the second stimulation (Sp_2) and the corresponding fraction of radioactivity released before the first period (Sp_1).

[c] Accumulation of total radioactivity in rat hypothalamic slices previously incubated for 30 min with 0.1 μM [^{3}H]-5-HT (specific activity, 12 Ci/mmol), determined at the end of the experiment.

The inhibitory effect of exogenous NE on [^{3}H]-5-HT overflow was completely antagonized in the presence of 0.1 μM RX 781094 (fig. 6).

***Alpha*-1 adrenoceptor agonists or antagonists fail to modify the electrically evoked release of [^{3}H]-5-HT.** The *alpha*-1 adrenoceptor agonist phenylephrine (0.1 and 1 μM) when added 20 min before S_2 failed to affect the stimulation-evoked release of [^{3}H]-5-HT (Table 4). At 1 μM, phenylephrine increased slightly but significantly the spontaneous outflow of radioactivity. Under the same experimental conditions, the *alpha*-1 adrenoceptor antagonist prazosin (0.1 μM) had no effect on [^{3}H]-5-HT overflow, while slightly increasing the spontaneous outflow of tritium (table 4). When prazosin (0.1 μM) was present in the medium 20 min before S_1 and maintained throughout the experiment, it failed to antagonize the inhibitory effect of 0.3 μM NE on the stimulation-evoked release of [^{3}H]-5-HT (table 4).

Effects of *alpha*-2 adrenoceptor antagonists on the electrically evoked release of [^{3}H]-5-HT in rat hypothalamic slices. Phentolamine at 0.1 μM did not affect the overflow of [^{3}H]-5-HT elicited by electrical stimulation (table 5), but effectively antagonized the inhibitory effects of clonidine (fig. 4), NE and 6F-NE (fig. 5). A higher concentration of phentolamine (1 μM) increased the electrically evoked release of [^{3}H]-5-HT (table 5) without affecting the spontaneous outflow of radioactivity (table 3). When cocaine (1 or 10 μM) was present throughout the experiment, the increase by 1 μM phentolamine of the electrically evoked release of [^{3}H]-5-HT was of the same magnitude as that obtained in the absence of cocaine (table 5). On the other hand, phentolamine (at 0.1 μM) failed to increase the electrically evoked release of [^{3}H]-5-HT in the absence or the presence of 1 or 10 μM cocaine (table 5).

The *alpha*-2 adrenoceptor antagonist RX 781094 when added 20 min before S_2 failed to modify the stimulation-evoked release of [^{3}H]-5-HT in rat hypothalamic slices in the range of concentrations tested (0.1–10 μM) (fig. 7). It is noteworthy that in this range of concentrations RX 781094 was effective in antagonizing the inhibitory effect of exogenous NE on [^{3}H]-5-HT overflow, as shown in figure 6. Therefore, effective blockade of presynaptic *alpha*-2 adrenoceptors present on serotonergic nerve endings in the rat hypothalamus did not *per se* result in an increase of the electrically evoked release of [^{3}H]-5-HT. These results support the view that endogenous NE released from noradrenergic neurons does not reach the presynaptic *alpha*-2 adrenoceptors located on serotonergic nerve endings in

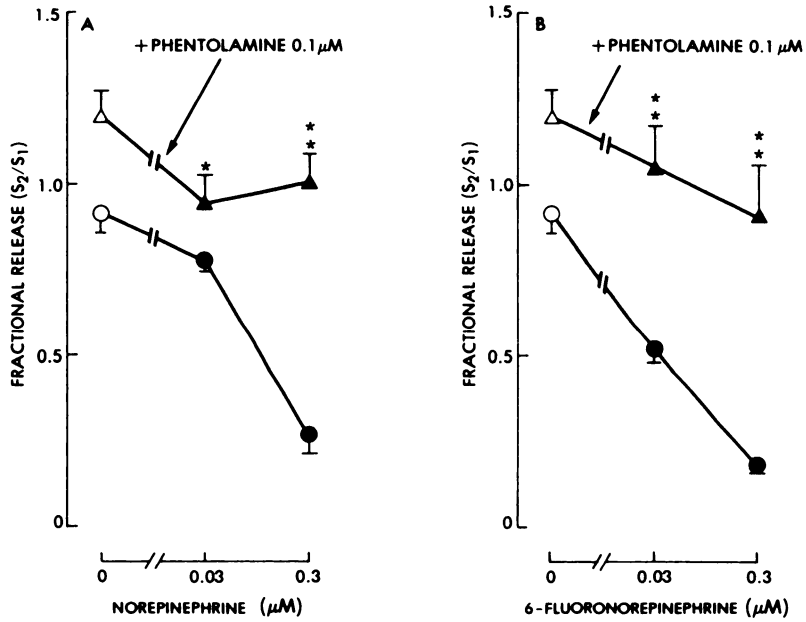

Fig. 5. Effect of phentolamine on the inhibition by NE and 6F-NE of the release of [^{3}H]-5-HT elicited by electrical stimulation from slices of the rat hypothalamus. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) expressed as the ratio obtained between S_2 and S_1 carried out within the same experiment. ○, control; △, control in the presence of phentolamine (0.1 μM) during S_1 and S_2. In A: ●, NE added before S_2; ▲, NE added before S_2 in the presence of phentolamine (0.1 μM) throughout the experiment. *Abscissa*: concentrations of NE (micromolar). NE in the concentrations indicated was added to the medium 20 min before S_2. In B: ●, 6F-NE added before S_2; ▲, 6F-NE added before S_2 in the presence of phentolamine (0.1 μM) throughout the experiment. *Abscissa*: concentrations of 6F-NE (micromolar). 6F-NE in the concentrations indicated was added to the medium 20 min before S_2. Each point represents mean values ± S.E.M. of at least six experiments per group. * $P < .05$; ** $P < .005$ when compared with the corresponding values obtained in the absence of phentolamine. NE, when present alone, significantly decreased [^{3}H]-5-HT release at 0.03 μM ($P < .05$) and 0.3 μM ($P < .001$) when compared with the control value in the absence of drugs. 6F-NE, when present alone, significantly decreased [^{3}H]-5-HT release at 0.03 μM ($P < .001$) and 0.3 μM ($P < .001$) when compared with the control value in the absence of drugs. The values of fractional release during the first period of electrical stimulation are shown in the legend of figure 4.

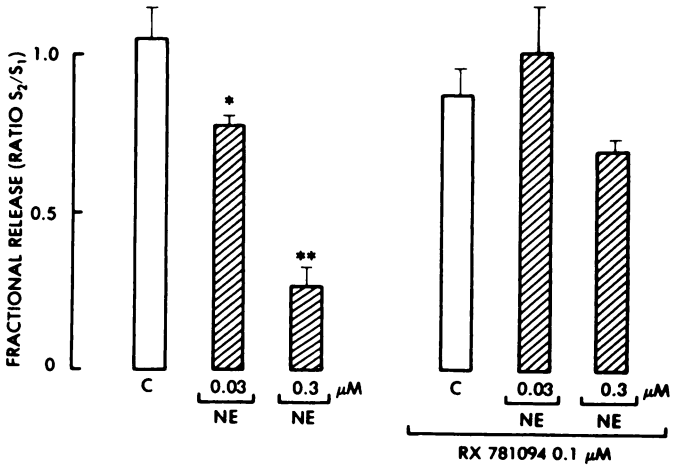

Fig. 6. Effect of RX 781094 on the inhibition by NE of [^{3}H]-5-HT overflow elicited by electrical stimulation from rat hypothalamic slices. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 2 msec, 20 mA) expressed as the ratio obtained between S_2 and S_1 carried out within the same experiment. NE was added 20 min before S_2. When used, RX 781094 (0.1 μM) was added 20 min before S_1 and remained present throughout the experiment. The mean values ± S.E.M. of at least three experiments per group are shown. The fractional release of radioactivity obtained in S_1 was 1.77 ± 0.25% of tissue stores (n = 8) in control (C) experiments and 2.31 ± 0.30% (n = 6) in the presence of RX 781094 (0.1 μM). * $P < .05$; ** $P < .01$ when compared with the corresponding control.

TABLE 4

Effects of *alpha*-1 adrenoceptor agonist and antagonist drugs on the electrically evoked [^{3}H]-5-HT overflow from rat hypothalamic slices

The radioactivity retained by the tissue after 130 min of superfusion was 40.1 ± 2.5 nCi/slice, n = 8 in control experiments. The radioactivity retained by tissues in all other experiments was not significantly different from this control value. Drugs in the concentrations indicated were added to the medium 20 min before S_2. In the second set of experiments, prazosin (0.1 μM) was added to the medium 20 min before S_1 and remained present throughout the experiment. Shown are mean values ± S.E.M. n, number of experiments. Note that the inhibitory effect of NE (0.3 μM) was of the same order of magnitude in the presence as well as in the absence of prazosin (0.1 μM).

Experimental Group	n	^{3}H-Transmitter Overflow[a] (Fractional release × 10^{-2})		Spontaneous Outflow (Fractional release × 10^{-2})
		S_1	Ratio[b] S_2/S_1	Ratio[c] Sp_2/Sp_1
Control	8	1.77 ± 0.25	1.05 ± 0.10	0.78 ± 0.01
Phenylephrine				
0.1 μM	6	1.69 ± 0.17	0.98 ± 0.16	0.78 ± 0.01
1 μM	4	1.79 ± 0.27	0.95 ± 0.15	0.94 ± 0.04*
Prazosin, 0.1 μM	3	1.90 ± 0.07	0.85 ± 0.10	0.98 ± 0.01*
NE, 0.3 μM	6	1.59 ± 0.23	0.68 ± 0.10*	0.74 ± 0.01
Prazosin, 0.1 μM (S_1–S_2)				
Control	6	1.74 ± 0.22	0.97 ± 0.10	0.78 ± 0.02
NE, 0.3 μM	5	1.21 ± 0.16	0.59 ± 0.05*	0.70 ± 0.01

[a] Fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 2 msec, 20 mA) 60 min (S_1) and 104 min (S_2) after the end of the incubation with [^{3}H]-5-HT.

[b] Ratio of fractional release obtained between S_2 and S_1.

[c] Ratio between the spontaneous outflow of radioactivity obtained during the 4 min preceding the second stimulation (Sp_2) and the corresponding fraction of radioactivity released spontaneously before the first stimulation period (Sp_1).

* $P < .05$ when compared with the corresponding control value.

a concentration high enough to modulate 5-HT-release under physiological conditions. The endogenous concentration of NE in the synaptic gap was increased by inhibition of the neuronal uptake of NE with cocaine and, under these conditions, the *alpha*-2 adrenoceptor antagonist RX 781094 was tested in a wide range of concentrations for its effects on the electrically evoked release of [^{3}H]-5-HT. Cocaine (1 μM) was added 20 min before S_1 and maintained in the superfusion medium throughout the experiment. Under these conditions the ratio S_2/S_1 was not different from unity (fig. 7). However, even in the presence of cocaine, the *alpha*-2 adrenoceptor antagonist RX 781094 (0.1 to 10 μM) failed to increase the stimulation-evoked release of [^{3}H]-5-HT in rat hypothalamic slices (fig. 7).

TABLE 5

Effect of phentolamine in the absence and in the presence of cocaine on the release of [^{3}H]-5-HT elicited by electrical stimulation from slices of the rat hypothalamus

Phentolamine was added to the medium 20 min before the second period of stimulation. When used, cocaine (1 or 10 μM) was added to the medium 20 min before S_1 and remained present throughout the experiment. Shown are mean values ± S.E.M. n, number of experiments.

Experimental Group	n	^{3}H-Transmitter Overflow[a] (Fractional release × 10^{-2})		Ratio S_2/S_1[b]
		S_1	S_2	
Control	8	1.77 ± 0.25	1.72 ± 0.17	1.05 ± 0.10
Phentolamine				
0.1 μM	5	2.07 ± 0.26	2.09 ± 0.33	1.05 ± 0.16
1 μM	9	1.75 ± 0.25	3.04 ± 0.43**	1.82 ± 0.30*
Cocaine, 1 μM (S_1–S_2)				
Control	6	1.49 ± 0.15	1.53 ± 0.19	1.03 ± 0.07
Phentolamine				
0.1 μM	5	1.44 ± 0.25	1.60 ± 0.31	1.10 ± 0.13
1 μM	6	1.86 ± 0.22	2.64 ± 0.14**	1.49 ± 0.14**
Cocaine 10 μM (S_1–S_2)				
Control	6	1.89 ± 0.22	1.99 ± 0.21	1.08 ± 0.09
Phentolamine				
0.1 μM	4	1.76 ± 0.17	1.99 ± 0.37	1.11 ± 0.13
1 μM	4	1.76 ± 0.11	3.25 ± 0.37**	1.83 ± 0.10**

[a] Fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 20 mA, 2 msec) 60 min (S_1) and 104 min (S_2) after the end of the incubation with [^{3}H]-5-HT.

[b] Ratio of fractional release obtained between S_2 and S_1.

* $P < .05$; ** $P < .01$ when compared with the corresponding control value.

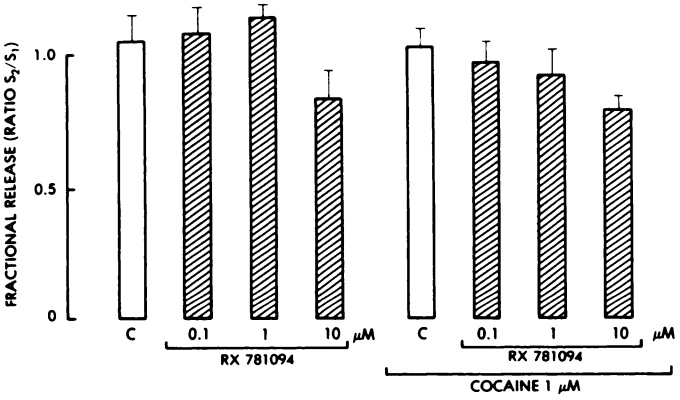

Fig. 7. Effect of RX 781094 in the presence or in the absence of cocaine on the stimulation-evoked release of [^{3}H]-5-HT from rat hypothalamic slices. *Ordinate*: fraction of the total tissue radioactivity released by a 2-min period of electrical stimulation (3 Hz, 2 msec, 20 mA) expressed as the ratio obtained between S_2 and S_1 carried out within the same experiment. RX 781094 at the concentrations indicated was added to the medium 20 min before S_2. When used, cocaine (1 μM) was added to the medium 20 min before S_1 and remained present throughout the experiment. The mean values ± S.E.M. of at least four experiments per group are shown. The fractional release of radioactivity obtained in S_1 was 1.77 ± 0.25% (n = 8) in control (C) experiments and 1.49 ± 0.15% (n = 6) in the presence of cocaine (1 μM).

Discussion

In rat hypothalamic slices and under the experimental conditions of our study, [^{3}H]-5-HT was selectively taken up and

stored in serotonergic nerve terminals. In support of this view, citalopram which selectively inhibits [^{3}H]-5-HT uptake in hypothalamic slices (Langer *et al.*, 1980) prevented the labeling of the hypothalamic slices with [^{3}H]-5-HT when used at a concentration of 1 μM. At this concentration of citalopram (1 μM) the accumulation of [^{3}H]NE in rat hypothalamic slices was not reduced. These results follow from the low potency of citalopram in inhibiting [^{3}H]NE uptake (Langer *et al.*, 1980). On the other hand, cocaine at 1 μM and desipramine at 0.3 μM reduced the uptake and retention of [^{3}H]NE but did not prevent the accumulation of [^{3}H]-5-HT in the slices of the rat hypothalamus. From these results, it can be concluded that [^{3}H]-5-HT accumulates in the slices of rat hypothalamus through the process of uptake and retention which takes place predominantly if not exclusively in serotonergic neurons. We can exclude the possibility that under our experimental conditions [^{3}H]-5-HT is taken up by the noradrenergic nerve terminals present in the hypothalamus, where it could behave as a false transmitter and be released by electrical stimuli.

Recently, it was shown that 6F-NE is a preferential *alpha*-2 adrenoceptor agonist (Shepperson *et al.*, 1981). The fact that NE and 6F-NE inhibited [^{3}H]-5-HT release evoked by electrical stimulation without increasing the spontaneous outflow of radioactivity indicates that both catecholamines, which are not preferentially taken up by serotonergic nerve endings, failed to displace [^{3}H]-5-HT stored in serotonergic nerve terminals. If, however, [^{3}H]-5-HT was to be partly stored in noradrenergic nerve terminals, then exposure to NE or 6F-NE should have increased substantially the spontaneous outflow of radioactivity.

Under our experimental conditions clonidine reduced the release of [^{3}H]-5-HT elicited by electrical stimulation in the low nanomolar range and behaved as an agonist with full intrinsic activity. In view of the pronounced inhibitory action of clonidine on central serotonergic transmission it is tempting to suggest that a reduction in 5-HT release could contribute to some of the centrally mediated pharmacological effects of this antihypertensive drug and in general to centrally acting *alpha*-2 adrenoceptor agonists.

The antagonism by phentolamine of the inhibitory effects of clonidine, NE and 6F-NE on the electrically evoked release of [^{3}H]-5-HT is compatible with the presence of inhibitory *alpha* adrenoceptors on the serotonergic nerve terminals of the rat hypothalamus, as already reported for the rat cortex (Göthert and Huth, 1980) and for the rat hippocampus (Frankhuyzen and Mulder, 1980) using NE as an *alpha* adrenoceptor agonist. The inhibitory *alpha* adrenoceptor on serotonergic nerve terminals has been characterized as having the pharmacological profile of the *alpha*-2 adrenoceptor subtype (Göthert *et al.*, 1981). In addition, these authors reported that the apparent pA_2 values for yohimbine against NE and clonidine were identical. As these results were obtained with yohimbine, a drug which by itself decreases the electrically evoked release of [^{3}H]-5-HT through an unknown mechanism of action (Göthert *et al.*, 1981), this conclusion remains to be re-examined with the use of other *alpha* adrenoceptor antagonists.

It was recently reported that in the frontal cortex of the rat, the *alpha* adrenoceptors which modulate the potassium-evoked release of [^{3}H]-5-HT may more closely resemble the *alpha*-1 rather than the *alpha*-2 subtype of adrenoceptors (Ennis, 1982). However, in the rat hypothalamus and under our experimental conditions, the *alpha*-1 agonist phenylephrine and the *alpha*-1 adrenoceptor antagonist prazosin were devoid of any effect on the electrically evoked release of [^{3}H]-5-HT. Moreover, the inhibitory effect of NE on [^{3}H]-5-HT release was not antagonized by prazosin, indicating that *alpha*-1 adrenoceptors are not present on serotonergic nerve endings of the rat hypothalamus. The discrepancy between our results and those obtained by Ennis (1982) may represent differences between the potassium and electrically evoked release of the transmitter or differences between the frontal cortex and the hypothalamus.

The *alpha* adrenoceptor antagonist, phentolamine, in a concentration which blocked the presynaptic inhibitory effects of clonidine, NE and 6F-NE on the overflow of [^{3}H]-5-HT, did not *per se* enhance the release of [^{3}H]-5-HT elicited by electrical stimulation. In a similar manner, the selective *alpha*-2 adrenoceptor antagonist, RX 781094, in a concentration which blocked the inhibition by NE of [^{3}H]-5-HT overflow, did not affect by itself the release of [^{3}H]-5-HT elicited by electrical stimulation. In contrast with these results, yohimbine and RX 781094 increased the overflow of [^{3}H]NE during electrical stimulation in slices of the rabbit hypothalamus. Similar results were obtained in slices of the rat hypothalamus prelabeled with [^{3}H]NE, ruling out the possibility that species differences may explain the lack of effect of *alpha*-2 antagonists on the electrically evoked release of [^{3}H]-5-HT in the rat. Phentolamine has also been shown to increase the electrically evoked release of [^{3}H]NE from slices of the rat occipital cortex (Pelayo *et al.*, 1980). The presynaptic *alpha*-2 adrenoceptors on noradrenergic nerve endings are therefore stimulated by NE released during nerve stimulation to autoinhibit its own release. Stimulation of presynaptic inhibitory *alpha*-2 adrenoceptors on noradrenergic nerves by released NE can be demonstrated in the absence and in the presence of cocaine (Langer and Dubocovich, 1981). In contrast with the physiological role played by presynaptic *alpha*-2 adrenoceptors on noradrenergic nerve terminals the presynaptic *alpha*-2 adrenoceptors on serotonergic nerve endings appear not to be involved in a physiological interaction of NE on serotonin release. In support of this view, exposure to 0.1 μM phentolamine did not increase [^{3}H]-5-HT overflow even when neuronal uptake of NE was inhibited with cocaine.

It could be argued that the facilitation by a higher concentration of phentolamine (1 μM) of the overflow of [^{3}H]-5-HT elicited by electrical stimulation could be related to blockade by phentolamine of the 5-HT autoreceptor. However, exogenous serotonin inhibits the electrically evoked release of [^{3}H]-5-HT when tested in the presence of citalopram and this action was not modified even in the presence of 10 μM phentolamine (C. Moret and S. Z. Langer, unpublished observations). Similarly, Göthert and Huth (1980) reported that 10 μM phentolamine does not block the presynaptic effects of 5-HT on the serotonergic autoreceptor when neuronal uptake is inhibited with paroxetine. Blockade by phentolamine of *alpha*-1 adrenoceptors can also be excluded because prazosin in a concentration as high as 0.1 μM did not affect the electrically evoked release of [^{3}H]-5-HT. The negative results obtained with phenylephrine and prazosin on [^{3}H]-5-HT release clearly exclude the possibility that *alpha*-1 adrenoceptors are involved in the presynaptic modulation of 5-HT release in the rat hypothalamus.

Therefore, the mechanism by which phentolamine at rather high concentrations increases the electrically evoked release of [^{3}H]-5-HT remains to be clarified. Our results do not support the view of Göthert and Huth (1980) that endogenously released

NE is able to activate the presynaptic inhibitory *alpha*-2 adrenoceptors on the 5-HT nerve endings. When the selective *alpha*-2 adrenoceptor antagonist RX 781094 was tested in the absence or in the presence of cocaine, it failed to affect the overflow of [^{3}H]-5-HT elicited by electrical stimulation (fig. 7).

Our results support the view that endogenous NE released by electrical stimulation in the hypothalamus does not reach the *alpha*-2 adrenoceptors located on serotonergic nerve endings in a concentration high enough to cause inhibition of 5-HT release. Similar conclusions were reached by Frankhuyzen and Mulder (1980) based on results obtained in the rat hippocampus.

It is of interest to note that the presynaptic modulation of [^{3}H]-5-HT release mediated by *alpha*-2 adrenoceptors in the rat cerebral cortex is not affected after 6-hydroxydopamine treatment or long-term administration of desipramine (Schlicker *et al.*, 1982). These results further support the view that the presynaptic *alpha*-2 adrenoceptors on serotonergic nerve terminals are not acted upon by NE released from noradrenergic neurons. It should be noted, however, that the presynaptic inhibitory *alpha*-2 adrenoceptors on serotonergic nerve terminals may be of pharmacological importance as they can be acted upon by exogenous *alpha*-2 adrenoceptors agonists to inhibit 5-HT release.

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