COMPARATIVE EFFECT OF SEROTONIN AGONISTS ON PRESYNAPTIC AND SOMATODENDRITIC AUTORECEPTORS OF SEROTONINERGIC NEURONS

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Antagonists of serotonin (hydroxytryptamine, 5-HT) modify the function of brain serotoninergic neurons in different ways. 5-HT agonists with a selective effect on type 1A serotonin receptors (R_{1A}-HT), buspirone for example, inhibit the function of 5-HT-ergic neurons, depressing their spike activity [10]. Activators of R_{1B}-HT inhibit the function of 5-HT-ergic brain neurons by depressing pulsed release of 5-HT by axon terminals [6]. Although depression of the function of 5-HT-ergic neurons of the midbrain nuclei raphe is known to lower the level of anxiety in animals [12], there is abundant evidence that in experimental models of anxiety states anxiolytic activity is exhibited only by 5-HT agonists which activate R_{1A}-HT, but not R_{1B}-HT [2]. The degree of selectivity of action of many 5-HT agonists on subtyptes of R_1-HT has been estimated by radioligand studies [5], but not in living functioning systems.

This paper gives the results of a comparative study of the effect of agonists on presynaptic and somatodendritic 5-HT autoreceptors in experiments on surviving brain slices.

EXPERIMENTAL METHOD

The effect of several 5-HT agonists was studied on spontaneous (basal) and electrically stimulated pulse release of ^{3}H-5-HT from thin slices [8] of cerebral cortex dorsal midbrain nuclei raphe, preincubated with ^{3}H-5-HT. Noninbred albino rats weighing 200 $\pm$ 30 g were used. Thin slices (200-250 μ) of cerebral cortex or dorsal midbrain nuclei raphe were preincubated for 30 min with 10^{-7} M ^{3}H-5-HT (specific activity 12 Ci/mmole; from "Amersham," England) in the presence of the monoamine oxidase inhibitor nialamide, washed three times with cold incubation medium (without ^{3}H-5-HT, 5 ml), and transferred to polyethylene grids of 0.5-ml perfusion chambers, located between two platinum electrodes. The slices were perfused at the rate of 0.5 ml/min with a solution of the following composition (in mM): NaCl – 122, KCl – 3.1, $CaCl_2$ – 1.3 $MgSO_4$ – 1.2 KH_2PO_4 – 0.4, $NaHCO_3$ – 2.5, glucose – 10, ascorbic acid – 1.14, nialamide – 1.25×10^{-5} M, pH 7.4. Five-minute portions of perfusion fluid were collected from the 30th minute after the beginning of perfusion, i.e., after spontaneous release of radioactive label had flattened out on a plateau. At the 45th and 60th minutes the slices were stimulated by square pulses (5 Hz, 2 msec, 12 mA) for 2 min (S_1 and S_2). The test substances were added to the medium 13 min before S_2, and the duration of perfusion with the substances was 15 min. Radioactivity of the samples was measured by means of a scintillation counter. The release of radioactive label during electrical stimulation was expressed as a coefficient of release. The numerical results were subjected to statistical analysis in the usual way. A series of ligands, which are activators of 5-HT receptors, was studied: 8-hydroxy-2-(di-N-propylamino)-tetraline (8-OH-DPAT); buspirone (TVX 5197); ipsapirone (generously provided by Dr. Georg Traber, Troponwerke, Cologne, West Germany) (TVX Q 7821);

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TABLE 1. Effect of Ligands (10^{-5} M) of Serotonin Receptors (R_1-HT) on Electrically Stimulated and Spontaneous Release of ^{3}H-5-HT by Cerebral Cortical Slices (A) and Slices of the Dorsal Nuclei Raphe (B) of Rats (M ± m)

Substance	Pulsed release S_2/S_1	Basal release in period		
		50/45 min	55/50 min	60/55 min
A. Control	0,98±0,06	0,97±0,10	0,97±0,08	0,98±0,11
Serotonin	0,21±0,10*	—	—	—
Ipsapirone	0,90±0,08	0,98±0,09	0,97±0,10	0,98±0,06
8-OH-DPAT	0,75±0,05*	0,98±0,11	0,98±0,04	0,98±0,10
Kampirone	0,72±0,03*	0,98±0,04	0,99±0,10	0,98±0,03
1,2-PP	0,70±0,04*	0,97±0,08	0,98±0,10	0,97±0,08
Kaplapirone	0,63±0,10*	0,98±0,15	0,97±0,08	0,98±0,12
Buspirone	0,51±0,03*	0,97±0,08	0,98±0,12	0,98±0,07
TFMPP	0,32±0,07*	0,94±0,12	0,96±0,10	0,96±0,14
B. Control	0,98±0,08	0,98±0,06	0,97±0,10	0,98±0,03
Serotonin	0,18±0,06*	—	—	—
TFMPP	0,86±0,12	0,99±0,05	0,98±0,08	0,98±0,06
Kaplapirone	0,60±0,10	0,98±0,06	0,97±0,10	0,98±0,07
1,2-PP	0,56±0,06*	0,98±0,08	0,98±0,06	0,98±0,10
Ipsapirone	0,47±0,04*	0,98±0,08	0,97±0,12	0,97±0,14
8-OH-DPAT	0,45±0,03*	0,97±0,10	0,98±0,04	0,98±0,03
Kampirone	0,42±0,08*	0,98±0,08	0,98±0,06	0,98±0,12
Buspirone	0,34±0,04*	0,98±0,10	0,98±0,12	0,98±0,08

Legend. Asterisk indicates values for which $p < 0.05$ compared with control.

kampirone and kaplapirone (synthesized in the Institute of Physico-Organic Chemistry and Carbon Chemistry, Academy of Sciences of the Ukrainian SSR, Donetsk). According to our data, all exhibit anxiolytic activity in models of anxiety in animals. For comparison we studied the effect of an active metabolite buspirone, namely 1-(2-pyrimidinyl)-piperazine (1,2-PP), which also has an anxiolytic action, and of 1-[3-(trifluoromethyl)phenyl]-piperazine (TFMPP), generously provided by Dr. Peter H. Hudson, which has an anxiogenic action [2], on pulsed release of ^{3}H-5-HT by the brain slices.

EXPERIMENTAL RESULTS

Electrical stimulation considerably increased the release of radioactive label from brain slices preincubated with ^{3}H-5-HT compared with spontaneous release. The ratios S_2/S_1 obtained on slices of cerebral cortex and dorsal midbrain nuclei raphe, incubated with labeled 5-HT, amounted to 0.98 ± 0.06 and 0.98 ± 0.08 respectively. 5-HT in a concentration of 10^{-5} M inhibited by 78.6% the pulsed release of radioactive label by electrically stimulated cortical slices and by 81.6% its release by slices of the dorsal nuclei raphe. The 5-HT agonist 8-OH-DPAT [5, 7, 9], acting selectively on R_{1a}-HT, and also the bispirone metabolite 1,2-PP and the compound kampirone in concentrations of 10^{-5} M, with no significant effect on basal release, moderately (by 23.5-28.5%) inhibited pulsed release of radioactive label by slices of rat cerebral cortex (Table 1A). Pulsed release of ^{3}H-5-HT from cortical slices was depressed by buspirone and haplapirone by 48 and 36% respectively; ipsapirone had no such effect. The most marked effect on release of labeled 5-HT from electrically stimulated slices of cerebral cortex was given by TFMPP, which in the same concentration, reduced release of the radioactive label by 67.3% (Table 1A). Meanwhile TFMPP did not inhibit release of ^{3}H-5-HT during electrical stimulation of slices of the dorsal midbrain nuclei raphe of rats (Table 1B). Kaplapirone and 1,2-PP, an active buspirone metabolite, had a moderate effect on pulsed release of the radioactive label, for they reduced ^{3}H-5-HT release by 38.7 and 42.8% respectively. Ipsapirone, 8-OH-DPAT, and kampirone had an equal effect on the process of ^{3}H-5-HT release by slices of the dorsal midbrain nuclei raphe, in response to electrical stimulation. In a concentration of 10^{-5} M these serotonin agonists inhibited ^{3}H-5-HT release by 52-57% (Table 1B). Buspirone, which in the same concentration reduced the release of ^{3}H-5-HT by 66%, had an effect approximately equal to that of serotonin (10^{-5} M) on ^{3}H-5-HT release from electrically stimulated slices of rat midbrain nuclei.

The results confirm the well-known property of serotonin, namely self-regulation of its pulsed release by terminals of 5-HT-ergic neurons through the intermediary of presynaptic autoreceptors [3], and at the same time they are evidence that pulsed release of 5-HT in somatodendritic synapses also is self-regulated by serotonin. It can be concluded from the experimental results that the inhibitory effect of 5-HT on its pulsed release in cerebral cortical slices is realized mainly through presynaptic R_{1B}-HT, since TFMPP, a selective activator of serotonin receptors of this type [2, 5], inhibit the outflow of ^{3}H-5-HT from

electrically stimulated cortical slices by the greatest degree, whereas the selective R_{1A}-HT agonist 8-OH-DPAT [2, 9], in the same concentration, is three times less effective, although it significantly (by 54%) inhibits the pulsed release of ^{3}H-5-HT in slices of the dorsal nuclei raphe. This last fact is in agreement with data in the literature on the localization of R_{1A}-HT in the soma or dendrites, but not in terminals of 5-HT-ergic axons [4, 11], where R_{1B}-HT are predominantly located [1].

While accepting the validity of the conclusion that R_{1A}-HT are located mainly in the soma or dendrites and R_{1B}-HT are located mainly in the soma or dendrites and R_{1B}-HT in terminals of 5-HT-ergic neurons, it can be asserted that ipsapirone exhibits affinity mainly toward R_{1A}-HT and has virtually no affinity for R_{1B}-HT, for it inhibits the release of radioactive label in slices of the nuclei raphe but does not affect presynaptic release of ^{3}H-5-HT by cortical slices. Kampirone has a very moderate action on presynaptic R_{1B}-HT, but at the same time it exhibits a marked effect on R_{1A}-HT and reduces pulsed release of ^{3}H-5-HT by slices of the nuclei raphe by 57%. Kaplapirone, on the other hand, with a moderate effect on release of radioactive lable by slices of the nuclei raphe, evidently has marked affinity for R_{1B}-HT, for it reduces the electrically stimulated release of ^{3}H-5-HT by 5-HT-ergic terminals only 50% less strongly than TFMPP. In agreement with the results of radioligand studies, which showed that buspirone exhibits high affinity for R_{1A}-HT, but as low affinity for R_{1B}-HT [5, 7], it inhibits pulsed release of ^{3}H-5-HT by electrically stimulated slices of the dorsal nuclei raphe less strongly than the other 5-HT agonists, but has an inhibitory effect also on pulsed release of ^{3}H-5-HT by terminals of 5-HT-ergic neurons in the cerebral cortex.

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