JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2001, 52, 2, 265—274

B. BOBULA, A. ZAHORODNA, M. BIJAK

DIFFERENT RECEPTOR SUBTYPES ARE INVOLVED IN THE SEROTONIN-INDUCED MODULATION OF EPILEPTIFORM ACTIVITY IN RAT FRONTAL CORTEX *IN VITRO*

Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

The frontal cortex is innervated by serotonergic terminals from the raphe nuclei and it expresses diverse 5-HT receptor subtypes. We investigated the effects of 5-HT and different 5-HT receptor subtype-selective agonists on spontaneous discharges which had developed in rat cortical slices perfused with a Mg^{2+}-free medium and the $GABA_A$ receptor antagonist picrotoxin. The frequency of synchronous discharges, recorded extracellularly in superficial layers (II/III) of the frontal cortex, was dose-dependently enhanced by 5-HT (2.5—40 μM). That excitatory effect was blocked by the 5-HT_2 receptor selective antagonist ketanserin. The $5\text{-HT}_{2A/2C}$ receptor-selective agonist DOI and the 5-HT_4 receptor agonist zacopride also increased the frequency of spontaneous discharges. In the presence of ketanserin, 5-HT decreased the discharge rate; a similar effect was observed when the 5-HT_{1A} receptor agonist 8-OH-DPAT or the 5-HT_{1B} receptor agonist CGS-12066B was applied. The 5-HT_3 receptor agonist m-CPBG was ineffective. In conclusion, 5-HT produces multiple effects on epileptiform activity in the frontal cortex via activation of various 5-HT receptor subtypes. The excitatory action of 5-HT, which predominates, is mediated mainly by 5-HT_2 receptors. The inhibitory effects can be attributed to activation of 5-HT_{1A} and 5-HT_{1B} receptors.

Key words: *5-HT, 5-HT*$_{1A}$ receptors, 5-HT_2 receptors, 5-HT_{1B} receptors, 5-HT_4 receptors cortical slice, synchronous activity.

INTRODUCTION

The frontal cortex is one of the brain areas receiving relatively dense serotonergic innervation from the raphe dorsalis. Cortical serotonin (5-hydroksytryptamine, 5-HT) receptors, mainly the 5-HT_2 subtype (1), have been implicated in the genesis of psychoses and in the antipsychotic action of drugs (2, 3). A number of *in vivo* and *in vitro* studies have been conducted to elucidate the effects of serotonin on cortical neurons and on the activity of

cortical networks; however, their results are still conflicting. Both excitation and inhibition of cortical neurons have been reported, being possibly due to activation of different 5-HT receptor subtypes (3—6). Diverse effects of 5-HT on the activity of cortical networks may also depend on the type of a neuron affected by 5-HT (glutamatergic vs. GABAergic) (7, 8). Several 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3, 5-HT_4) have been identified in the frontal cortex, among which 5-HT_{2A} receptors predominate (1). High densities of both 5-HT_{2A} binding sites and transcripts have been found in the cortex (8, 9). Cortical 5-HT_{2A} receptors have been hypothesized to be the primary site of action of hallucinogens; moreover, they may be critically involved in the actions of many atypical antipsychotics and some antidepressants (2). The majority of 5-HT_{2A} immunoreactive cells display pyramidal morphology, and the very dense labeling of apical dendrites is particularly prominent (8). These findings suggest that 5-HT affects principal glutamatergic cortical neurons. However, other studies indicate that cortical interneurons receive dense serotonergic innervation (10) and that mainly interneurons express 5-HT_{2A} receptors (11, 12). There are also some discrepancies between electrophysiological studies which demonstrate that serotonin induces release of glutamate or GABA from cortical neurons through 5-HT_{2A} receptors (3, 13).

The present study was designed to assess the action of 5-HT on synchronous, epileptiform activity of cortical neurons by examining effects of different subtype-selective 5-HT receptor agonists on field potentials which develop in a Mg^{2+}-free solution in rat frontal cortex slices. The removal of Mg^{2+} block from NMDA receptors and the reduced membrane surface charge screening enhance synaptic excitatory potentials and neuronal excitability and result in synchronous, spontaneous discharges of cortical neurons (14, 15). Such discharges represent a network-dependent activity, and their frequency is sensitive to both the direct action on bioelectrical properties of cortical neurons and on synaptic transmission. We employed that model because the population activity in an interconnected network of neurons is usually very sensitive to pharmacological manipulations, since small effects sum up at different functional targets which contribute to this activity. The $GABA_A$ antagonist picrotoxin was employed to block fast synaptic inhibition, to study the effects of 5-HT on an isolated excitatory synaptic network.

MATERIALS AND METHODS

Experiments

Male Wistar rats (120—150 g) were decapitated, their frontal cortices were dissected and cut into 400—450 μm-thick slices. The slices were kept in a gassed medium (95% O_2 5% CO_2) consisting of (in mM): 127 NaCl, 2 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 KH_2PO_4, 24 $NaHCO_3$ and

10 glucose. A single slice was transferred to the recording chamber (volume of 1 ml) and superfused at a rate of 1.5 ml/min with a medium devoid of magnesium ions and with added picrotoxin (30 μM) at 32°C. Spontaneous field potentials were recorded in superficial layers of the cortex (II/III) (an Axoprobe amplifier, Axon Instruments, USA) using glass micropipettes filled with 2 M NaCl (2—5 MΩ). The spontaneous potentials were displayed on a chart recorder (Gould TA 240) and stored on a PC computer (CED 1401 interface, SIGVAG software, Cambridge Electronics, UK) for a further analysis. When the frequency of discharges was stable for 30 min, 5-HT or one of the 5-HT receptor subtype-selective agonists was bath-applied for 10 min. The frequency of discharges, the number of afterpotentials per discharge and the duration of a discharge were measured before and during application, and upon the washout. The effects were expressed as a percentage of baseline control. A statistical analysis was carried out using Student's t-test.

Drugs

The drugs used: 5-hydroxytryptamine creatinine sulfate (5-HT; Sigma USA), (±)-2-dipropylamino- 8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT; RBI); 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[1,2-a]quinoxaline maleate (CGS-12066B maleate, RBI), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride ((±)-DOI hydrochloride, RBI), I-(m-Chlorophenyl)-biguanide hydrochloride (m-CPBG, RBI), 4-amino-5-chloro-2-methoxy--substituted benzamide ((R,S)zacopride, generously donated by Delalande, France), 3-[2[4-(4--fluorobenzoyl)-1-piperdinyl]ethyl]-2,4(1H,3H)-quinazolinedione tartrate (ketanserin tartrate, RBI), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; RBI), DL-2-amino-4-methyl-5-phosphono--3-pentanoic acid (CGP 37849; kindly donated by CIBA-GEIGY, Basel, Switzerland).

RESULTS

Characteristic of field potentials recorded in cortical slices

Spontaneous epileptiform potentials were recorded in cortical slices within 20—40 min of superfusion with an Mg^{2+}-free medium. Simultaneous recordings from deep and superficial layers showed that those events occurred in parallel throughout all layers of the cortex, but the most pronounced signals were recorded from superficial layers (II/III). Discharges occurred at a frequency ranging from 0.01 to 0.7 Hz (0.04 ± 0.002, $n = 56$; *Fig. 1*). Each discharge consisted of a field potential with superimposed afterpotentials (*Figs 2A2, 4A2*). Because the number of afterpotentials per discharge was correlated with the duration of a discharge (*Fig. 1C*), the number of afterpotentials was employed in further analyses. The number of afterpotentials per discharge varied between slices, but was fairly constant in a slice, being inversely related with the frequency of discharges (*Fig. 1B*). The competitive NMDA receptor antagonist CGP 37849 (1 μM) reversibly suppressed spontaneous discharges, while the non-NMDA receptor antagonist CNQX (5 μM) decreased their frequency (by $40 \pm 6\%$, $n = 6$).

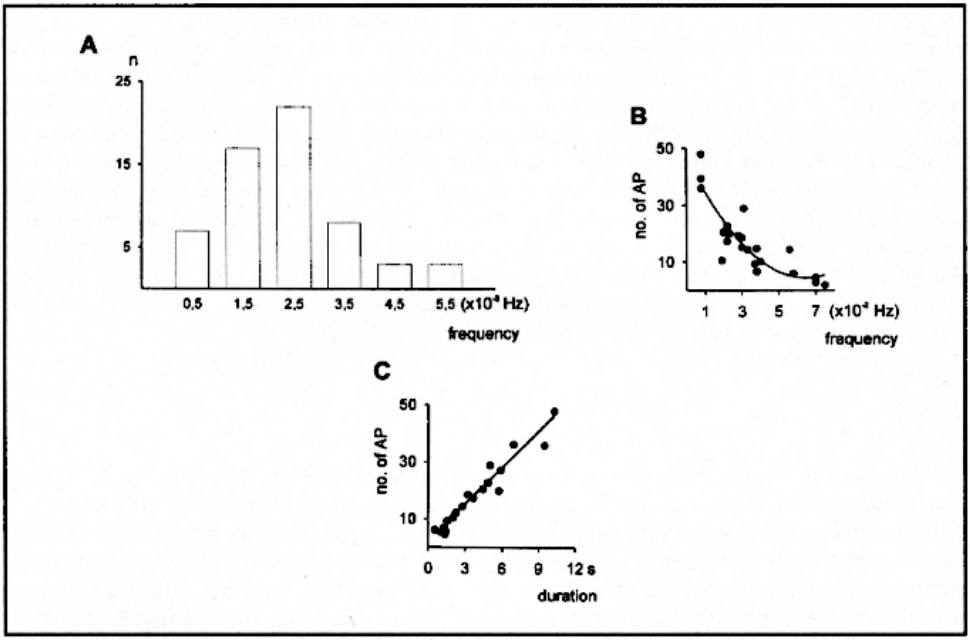

Fig. 1. Plots characterizing the synchronous activity recorded from frontal cortex slices. (A) Distribution of discharge frequencies in all the slices studied (n = 56). (B) The relationship between the number of afterpotentials (AP) and the frequency of synchronous discharges. (C) The correlation between the duration of discharges and the number of afterpotentials.

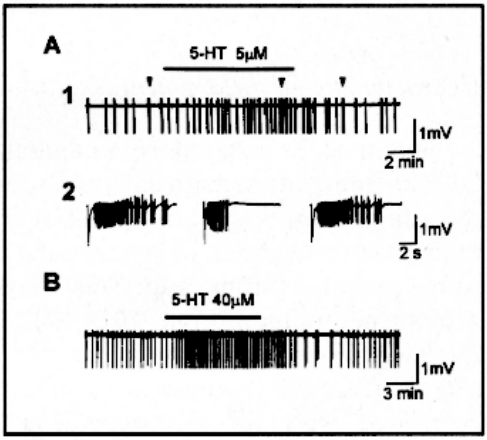

Fig 2. Effects of 5-HT on spontaneous discharges recorded from a frontal cortex slice. (A) 5-HT (5 μM) reversibly increased the frequency of discharges (1) and decreased the number of afterpotentials per discharge, (shown in A2, at a faster chart speed). Recordings shown in A2 were taken at the points marked by arrowheads in A1. (B) 5-HT (40 μM), applied to another slice, had a strong excitatory effect followed by inhibition of the synchronous activity. The time of 5-HT application is indicated with bars above traces.

Effects of 5-HT agonists

The bath-applied 5-HT produced an increase in the discharge rate in the majority of the slices tested (*Fig. 2*; *Table 1*). That effect was reversible, dose-dependent, and was accompanied with a decrease in the number of

afterpotentials per discharge (*Figs 2, 3*). In the presence of serotonin, the relation between the number of afterpotentials and the frequency of discharges remained the same as under baseline (control) conditions (*Fig. 3C*). The excitatory action of 5-HT was not desensitized throughout the application (10 min, *Fig. 2*). When high concentrations (40 μM) of 5-HT were applied, the inhibition of discharges was evident during the washout period (*Fig. 2B*). In the presence of the 5-HT_2 receptor antagonist ketanserin (1 μM), 5-HT (5 μM) decreased the frequency of spontaneous discharges (by $27 \pm 4\%$, $n = 14$). At the concentration used, ketanserin had no effect of its own on the discharges.

Table 1. Effects of 5-HT on the frequency of spontaneous discharges recorded from the frontal cortex.

5-HT (μM)	excitation (% n)	inhibition (% n)	no effect (% n)	n
2.5	70	0	30	10
5	76	0	24	25
10	83	0	7	6
20	64	27	9	11
40	100	0	0	4

Data are presented as a percentage of slices which were excited, inhibited or not affected by 5-HT (reaction = a statistically significant change in the discharge rate); n refers to the total number of the slices tested.

The 5-$HT_{2A/2C}$ agonist DOI (0.5—10 μM) mimicked the excitatory effect of 5-HT, but in most cases the number of afterpotentials per discharge was not altered (*Fig. 4*). The dose-dependence of the DOI-induced increase in the discharge frequency demonstrated that the maximal effect of DOI was weaker than that of 5-HT. Also zacopride (5 μM), a 5-HT_4 receptor agonist, increased the discharge rate ($141.3 \pm 7.8\%$, $P \leqslant 0.01$, $n = 6$); however, the number of afterpotentials per discharge was not changed. The 5-HT_{1A} selective agonist 8-OH-DPAT (1—5 μM) decreased the discharge rate without affecting the number of afterpotentials (*Fig. 5*). In the range of concentrations used in this study the inhibitory effect of 8-OH-DPAT displayed no dose-dependence (*Fig. 5B*). The 5-HT_{1B} receptor agonist CGS-12066B (5 μM) slightly decreased the rate of discharges ($80.3 \pm 4.6\%$, $P \leqslant 0.05$, $n = 6$), but had no effect on the number of afterpotentials. The 5-HT_3 receptor agonist m-CPBG (5 μM) was ineffective ($n = 4$).

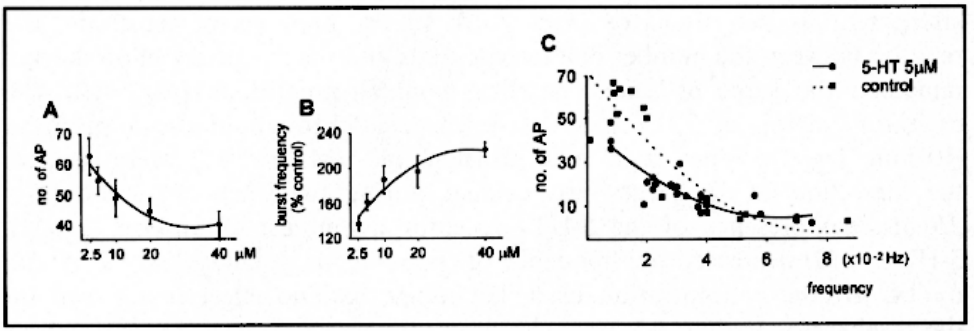

Fig. 3. Dose-dependence of the effects of 5-HT on the number of afterpotentials (AP), shown in A and the frequency of discharges, shown in B. (C) The plot showing a relationship between the number of afterpotentials (AP) and the frequency of synchronous discharges under control conditions and during application of 5-HT.

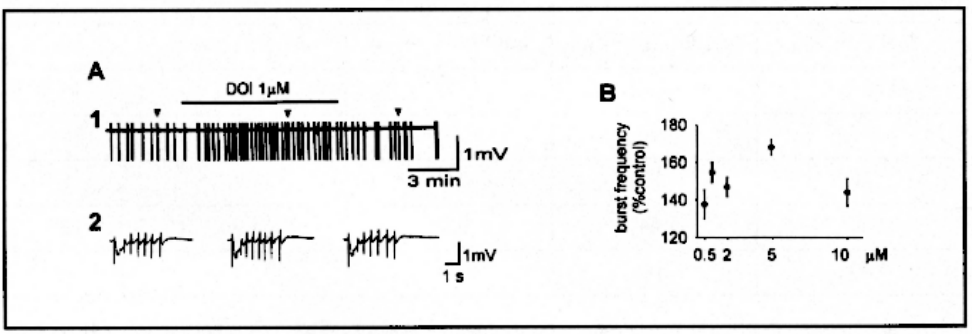

Fig. 4. Effects of the 5-HT_2 receptor agonist DOI on synchronous discharges. (A) A representative recording of the DOI-induced increase in the frequency of discharges (A1), without alterations in discharge duration (A2). (B) Dose-dependence of the effect of DOI on the discharge rate (n = 6—12).

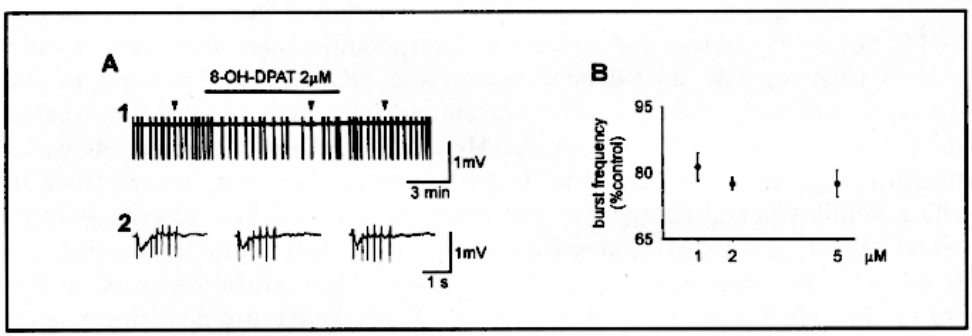

Fig. 5. 8-OH-DPAT (2 μM) attenuated the rate of occurrence of spontaneous discharges (A1), having no effect on the number of afterpotentials (A2). The time of application of 8-OH-DPAT is indicated with a bar above the trace. (B) The effect of 8-OH-DPAT on the discharge rate (n = 5—8) shows no dose-dependence over the range of concentrations studied.

DISCUSSION

The results of the present study show that actions of 5-HT on cortical epileptiform discharges are complex and involve different 5-HT receptor subtypes. The main effect of 5-HT on the synchronous activity that develops in a network of glutamatergic cortical cells incubated in an Mg^{2+}-free solution is an increase in the discharge rate which is mediated mainly via 5-HT_2 receptors, since the 5-HT-induced excitation is blocked by the selective 5-HT_2 receptor antagonist ketanserin. Electrophysiological data suggest that cortical 5-HT_{2A} receptors enhance the glutamate-induced excitatory postsynaptic potentials at apical dendrites of pyramidal cells (2, 3). Furthermore, it has been shown that activation of 5-HT_2 receptors can induce a depolarizing response in cortical neurons (16). These findings correspond well with the present observation that both 5-HT and DOI enhance spontaneous discharges in cortical slices. While the effects of 5-HT — especially at higher concentrations — were very pronounced, the excitatory action of DOI was weaker. This finding is in line with the data demonstrating that DOI is a partial agonist at cortical 5-HT_{2A} receptors (7, 17).

Also other 5-HT receptors may be involved in the observed increase in the discharge rate in response to 5-HT application, as indicated by the excitatory action of zacopride, a 5-HT_4 receptor agonist (1). So far, electrophysiological effects of 5-HT_4 receptor activation have been studied mainly in the hippocampus (18). In hippocampal pyramidal cells, the 5-HT-induced decrease in slow afterhyperpolarization (sAHP) that follows a train of action potentials was attributed to 5-HT_4 receptor activation (1, 18). Such a mechanism may be responsible for an increase in the discharge rate, observed in our experiments. Although the sAHP time course does not correlate well with the interval between spontaneous discharges in the cortex, the role of sAHP in determining the rate of synchronous activity has been suggested in modeling and experimental studies (19, 20, 21).

Excitatory responses to 5-HT can also be mediated by 5-HT_3 receptors via an opening of their cationic channel. These receptors are generally characterized by a fast, rapidly desensitizing reaction (1). Therefore, considering the slow mode of application in our experiments, 5-HT_3 receptors are likely to become desensitized, which may be the reason why no effect of the selective 5-HT_3 agonist m-CPBG was observed.

The present study has demonstrated that 5-HT enhances the synchronous activity induced in an Mg^{2+}-free solution in the frontal cortex *in vitro*, which is opposite to the inhibitory effects of 5-HT described in the entorhinal cortex and hippocampus (22, 23). An inhibitory reaction to 5-HT has been reported in a wide variety of neurons, and such responses have been generally attributed to activation of 5-HT_{1A} receptors (1, 18). The ionic basis for the 5-HT_{1A}-receptor

mediated inhibition is the opening of K^+ channels and hyperpolarization of the cell membrane. Binding studies clearly show that 5-HT_{1A} receptors predominate in the hippocampus and entorhinal cortex but are less densely expressed in the frontal cortex (1). In rat cortex it has been shown that the majority of pyramidal neurons coexpress 5-HT_2 and 5-HT_{1A} receptors (4). In line with the latter results, the 5-HT_{1A} receptor agonist 8-OH-DPAT had an inhibitory effect on the synchronous activity elicited in frontal cortex slices.

The 5-HT_{1B} receptor subtype is considered to be located presynaptically and has often been implicated in inhibition of the neurotransmitter release (1). *In vitro* studies have shown that — while acting on presynaptic 5-HT_{1B} receptors — serotonin inhibits glutamatergic synaptic transmission in the cingulate cortex (6, 24). Having shown that the 5-HT_{1B} agonist CGS-12066B diminished the frequency of spontaneous discharges, our results suggest that presynaptic 5-HT_{1B} receptors are likely to decrease glutamate release from intracortical glutamatergic connections in rat frontal cortex.

In our study 5-HT agonists affected mainly the frequency of spontaneous discharges, while the duration of individual discharges was in most cases unchanged. In fact, this finding implies some effects on the duration of discharges, since frequency and duration are inversely related. While increasing the discharge rate, only 5-HT simultaneously decreased the number of afterpotentials in a discharge (which is a measure of discharge duration). The mechanisms involved in regulation of the discharge duration have not been well understood so far, but both glutamate-mediated synaptic potentials and intrinsic voltage-dependent properties of neurons seem to be involved. Different potassium conductances take part in limiting the number of afterpotentials. Some of these potassium conductances are affected by 5-HT_2 and 5-HT_4 receptors. It is likely that DOI and zacopride increase the frequency of discharges without changing their duration due to blockade of some potassium channels, while such an effect is less pronounced when 5-HT is applied, since diverse actions depending on activation of different receptor subtypes are involved (4).

A number of experiments in which *in vivo* microiontophoretic methods are used and 5-HT is applied *in vitro* to cortical slices have shown how 5-HT affects neuronal behavior in the cortex. While the predominant 5-HT effect reported by *in vivo* studies is inhibition of the spontaneous activity of pyramidal neurons, *in vitro* results suggest that 5-HT has mainly an excitatory action in the cortical neuronal circuitry (18). This discrepancy may be partly attributed to strong enhancement of the synaptic inhibition *in vivo*, via 5-HT_3 and 5-HT_2 receptors, while inhibitory interneurons and their connections are partly disrupted in an *in vitro* slice preparation.

5-HT receptors comprise a complex family, since 7 major subgroups have been defined which are further subdivided into a number of subtypes (10).

Numerous 5-HT receptor subtypes are expressed in the cerebral cortex. Studies into the effects of 5-HT on cortical neurons are likely to yield conflicting results reflecting the diversified distribution and density of 5-HT receptor subtypes in different cortical areas and on various types of cortical neurons.

Acknowledgements: This study was supported by a statutory grant from the State Committee for Scientific Research (KBN), awarded to the Institute of Pharmacology.

REFERENCES

1. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* 1999; 38: 1083—1152.
2. Marek GJ, Aghajanian GK. The electrophysiology of prefrontal serotonin systems: therapeutic implications for mood and psychosis. *Biol Psychiatry* 1998; 44: 1118—1127.
3. Marek GJ, Wright RA, Schoepp DD, Monn JA, Aghajanian GK. Physiological antagonism between 5-hydroxytryptamine(2A) and group II metabotropic glutamate receptors in prefrontal cortex. *J Pharmacol Exp Ther* 2000; 292: 76—87.
4. Araneda R, Andrade R. 5-Hydroxytryptamine2 and 5-hydroxytryptamine 1A receptors mediate opposing responses on membrane excitability in rat association cortex. *Neuroscience* 1991; 40: 399—412.
5. Schmitz D, Gloveli T, Empson RM, Heinemann U. Potent depression of stimulus evoked field potential responses in the medial entorhinal cortex by serotonin. *Br J Pharmacol* 1999; 128: 248—254.
6. Tanaka E, North RA. Cocaine enhancement of the action of 5-hydroxytryptamine in rat cingulate cortex *in vitro*. *Neurosci Lett* 1993; 163: 50—52.
7. Marek GJ, Aghajanian GK. LSD and the phenethylamine hallucinogen DOI are potent partial agonists at 5-HT_{2A} receptors on interneurons in rat piriform cortex. *J Pharmacol Exp Ther* 1996; 278: 1373—1382.
8. Willins DL, Deutch AY, Roth BL. Serotonin 5-HT_{2A} receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse* 1997; 27: 79—82.
9. Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L. Comparative localization of serotonin 1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J Comp Neurol* 1995; 351: 357—373.
10. Smiley JF, Goldman-Rakic PS. Serotonergic axons in monkey prefrontal cerebral cortex synapse predominantly on interneurons as demonstrated by serial section electron microscopy. *J Comp Neurol* 1996; 367: 431—443.
11. Morilak DA, Garlow SJ, Ciaranello RD. Immunocytochemical localization and description of neurons expressing serotonin 2 receptors in the rat brain. *Neuroscience* 1993; 54: 701—717.
12. Morilak DA, Somogyi P, Lujan-Miras R, Ciaranello RD. Neurons expressing 5-HT_2 receptors in the rat brain: neurochemical identification of cell types by immunocytochemistry. *Neuropsychopharmacology* 1994; 11: 157—166.
13. Sheldon PW, Aghajanian GK. Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: evidence for the involvement of a 5-HT_2-activated interneuron. *Brain Res* 1990; 506: 62—69.
14. Jones RS, Heinemann U. Synaptic and intrinsic responses of medical entorhinal cortical cells in normal and magnesium-free medium *in vitro*. *J Neurophysiol* 1988; 59: 1476—1496.

15. Traub RD, Jefferys JG, Whittington MA. Enhanced NMDA conductance can account for epileptiform activity induced by low Mg^{2+} in the rat hippocampal slice. *J Physiol (Lond)* 1994; 478: 379—393.
16. Newberry NR, Footitt DR, Papanastassiou V, Reynolds DJM. Actions of 5-HT on human neocortical neurones *in vitro*. *Brain Res* 1999; 833:93—100.
17. Sanders-Bush E, Burris KD, Knoth K. Lysergic acid diethylamide and 2,5-dimethoxy--4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. *J Pharmacol Exp Ther* 1988; 246: 924—928.
18. Aghajanian GK. Electrophysiology of serotonin receptor subtypes and signal transduction pathways. In: Psychopharmacology: The fourth generation of progress. Floyd E. Bloom and David J.Kupfer, ed. New York: Raven Press. Ltd. 1995; 451—460.
19. Chamberlin NL, Dingledine R. Control of epileptiform burst rate by CA3 hippocampal cell afterhyperpolarizations *in vitro*. *Brain Res* 1989; 492: 337—346.
20. Traub RD, Dingledine R. Model of synchronized epileptiform bursts induced by high potassium in CA3 region of rat hippocampal slice. Role of spontaneous EPSPs in initiation. *J Neurophysiol* 1990; 64: 1009—1018.
21. Rutecki PA. Noradrenergic modulation of epileptiform activity in the hippocampus. *Epilepsy Res* 1995; 20: 125—136.
22. Schmitz D, Empson RM, Gloveli T, Heinemann U. Serotonin blocks different patterns of low Mg^{2+}-induced epileptiform activity in rat entorhinal cortex, but not hippocampus. *Neuroscience* 1997; 76: 449—458.
23. Salgado-Commissariat D, Alkadhi KA. Serotonin inhibits epileptiform discharge by activation of $5\text{-}HT_{1A}$ receptors in CA1 pyramidal neurons. *Neuropharmacology* 1997; 36: 1705—1712.
24. Schmitz D, Gloveli T, Empson RM, Draguhn A, Heinemann U. Serotonin reduces synaptic excitation in the superficial medial entorhinal cortex of the rat *via* a presynaptic mechanism. *J Physiol (Lond)* 1998; 508: 119—129.

Received: January 24, 2001
Accepted: April 5, 2001

Author's address: Maria Bijak, Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland. tel.: (048) 12 637-40-22, fax: (048) 12 637-45-00.
E-mail: nfbijak@cyf-kr.edn.pl