LITERATURE CITED

1. N. A. Bondarenko, V. A. Kamysheva, M. F. Mineeva, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 1, 20 (1981).
2. R. U. Ostrovskaya and S. S. Trofimov, Byull. Éksp. Biol. Med., No. 2, 170 (1984).
3. R. U. Ostrovskaya, S. S. Trofimov, N. M. Smol'nikova, and Yu. V. Burov, All-Union Symposium on Mediators in the Genetic Regulation of Behavior [in Russian], Novosibirsk (1986).
4. S. Tuneva, Ts. Tencheva, N. Tyutnelkova, and M. Nikolova, Mediko-Biol. Inform., No. 6, 8 (1980).
5. S. L. Shelekhov and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 8, 59 (1983).
6. S. H. Barondes and H. D. Cohen, Science, 160, 556 (1968).
7. H. D. Hendersen, J. Psychol., 75, No. 19 (1970).
8. L. K. Rägo, L. H. Allikmets, and A. M. Zarkovsky, Naunyn-Schmiederberg's Arch. Pharmakol., 318, 36 (1981).
9. R. H. Roth, G. B. Doherty, and J. B. Walters, Brain Res., 189, 556 (1980).
10. G. Schmaltz and P. Marcant, Physiol. Behav., 30, No. 6, 845 (1983).

SPINAL GANGLIONIC NEURONS OF RATS: A MODEL FOR THE STUDY OF CENTRAL SEROTONIN RECEPTORS

I. I. Abramets, I. V. Komissarov, and I. M. Samoilovich

UDC 612.822.014.46:[615.357:577.175.823].08

KEY WORDS: spinal ganglion; neurons; serotonin receptors.

Radioligand investigations have revealed two populations of serotonin receptors (SR) in brain membranes, of which one (S_2R) is labeled by ^{3}H-spiroperidol, the other (S_1R) by ^{3}H-serotonin; ^{3}H-lysergic acid diethylamide (LSD) labels both SR populations [9]. More recent studies have shown that S_1R can be subdivided into several subtypes [4, 7].

The results of electrophysiological investigations confirm this view that there are two populations of SR. The action of serotonin and stimulation of the nuclei raphe intensified spontaneous discharges or depolarization of neurons in the frontal cortex and dorsal hippocampus and of spinal motoneurons [1, 5, 10]; these effects, moreover, were abolished by LSD, cyproheptadine, methysergide, and other substances displacing ^{3}H-spiroperidol from its binding, methylsergide, and other substances displacing ^{3}H-spiroperidol from its binding with S_2R. However, serotonin antagonists did not affect serotonin-induced inhibition of neuronal activity in the frontal cortex, hippocampus, amygdala, and hypothalamus [6, 10]. These facts are evidence that S_1R mediate inhibition and S_2R mediate excitation of functions of central neurons. The concrete mechanisms of serotonin-inducted excitation or inhibition of nerve cell functions have not been adequately studied.

The investigation described below showed that S_1R and S_2R are represented in membranes of rat spinal ganglionic neurons, and the mechanisms of the excitatory and inhibitory action of serotonin on these nerve cells also were studied.

EXPERIMENTAL METHOD

Experiments were carried out on isolated lumbar spinal ganglia of adult rats. After isolation and removal of the meninges a spinal ganglion was fixed to the floor of a perfusion chamber, where it was superfused with salt solution of the following composition (in mM): NaCl 124, KCl 2, K_2HPO_4 1.25, $NaHCO_3$ 25, $CaCl_2$ 2, $MgSa_4$ 1, glucose 10. The solution was aerated intensively with a mixture of 95% O_2 + 5% CO_2; the pH of the solution was 7.4. The experiments were carried out at a temperature of 27°C.

Department of Pharmacology, M. Gor'kii Donetsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR D. A. Kharkevich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 8, pp. 172-174, August, 1988. Original article submitted August 4, 1987.

 0007-4888/88/0008-1112$12.50

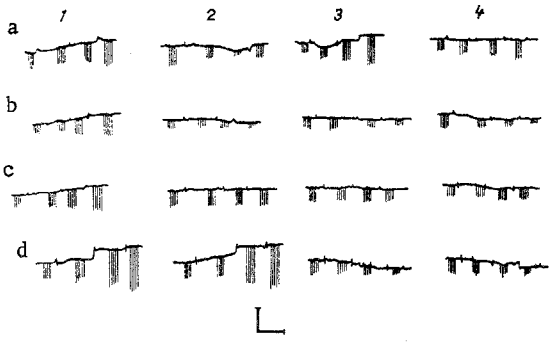

Fig. 1

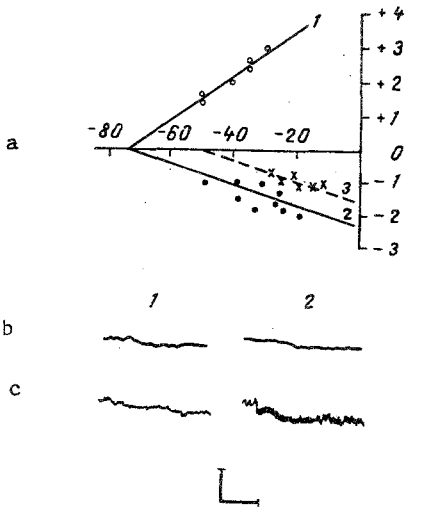

Fig. 2

Fig. 1. Pharmacological analysis of serotonin-induced responses of rat spinal ganglionic neurons. Changes in membrane potential and input resistance under the influence of serotonin before (a: 1-4; b, c, and d: 1 and 3) and 15 min after beginning of superfusion of ganglion with solution containing methylsergide (b: 2 and 4) or amitriptyline (c: 2 and 4) in a concentration of 10^{-6} M, or propranolol (d: 2 and 4) in a concentration of $3 \cdot 10^{-6}$ M. Here and in Fig. 2, calibration: 5 mV, 1 min.

Fig. 2. Analysis of ionic nature of serotonin-induced responses of rat spinal ganglionic neurons: a) dependence of magnitude (in mV) of serotonin-induced depolarization (1) or hyperpolarization (2) response on membrane potential level in solution containing K^+ in concentration of 3.25 mM (1, 2) and 10 mM (3); b, c) hyperpolarization responses of neurons to serotonin in absence (1) and in presence of 5 mM TEA (b, 2) or 0.1 mM 4-aminopyridine (c, 2).

The membrane potential and input resistance of the neurons were recorded by means of glass microelectrodes, filled with 3M KCl solution, the resistance of whose tip was 20-60 MΩ. The input resistance of the membrane was determined by passing hyperpolarizing pulses of current with a strength of 0.02-0.1 nA, duration 300 msec, and frequency once every 5 sec, through the recording microelectrode by means of a bridge circuit. For long shifts of membrane potential of the neurons, an inward or outward current was passed through the recording microelectrode by means of the bridge circuit.

After adaptation of the ganglion to the salt solution and penetration of the neurons, the ganglion was superfused with a solution containing serotonin in a concentration of $3 \cdot 10^{-5}$ M and the test parameters were recorded. Methylsergide, amitriptyline, and propranolol, in concentrations of 10^{-6}-$3 \cdot 10^{-6}$ M, were used as serotonin antogonists. The ganglion was superfused with salt solution containing the antagonists for 15 min.

EXPERIMENTAL RESULTS

Superfusion of the spinal ganglion with salt solution containing serotonin ($3 \cdot 10^{-5}$ M) induced weak depolarization (1-5 mV) in one-third of the neurons, accompanied by reduction of conductivity of the membrane. Some neurons (sbout 30%) responded to serotonin by hyperpolarization, the amplitude of which did not exceed 5 mV, and by an increase in membrane conductivity by 20-40%. In the remaining 30% of neurons mixed reactions were observed by serotonin: hyperpolarization followed by depolarization, or weak hyperpolarization with an increase in input resistance (Fig. 1).

Depolarization and the increase in input resistance of the spinal ganglionic neurons induced by serotonin were significantly reduced by methysergide and amitriptyline, which have affinity for S_2R [8]. This suggests that serotonin-induced depolarization of spinal neurons is effected through S_2R. Methylsergide and amitriptyline had no significant effects on serotonin-induced hyperpolarization of the neurons. Neither depolarization nor

hyperpolarization responses of the spinal ganglionic neurons were altered by propranolol (Fig. 1). Since according to the results of radioligand investigations propranolol does not interact with S_2R, but has high affinity for the S_{1B} subtype of serotonin receptors [4], there is reason to suppose that serotonin-induced hyperpolarization of spinal ganglionic neurons is effected through the S_{1A} subtype of serotonin receptors.

Investigation of the ionic dependence of serotonin-induced responses of the neurons at diffetent membrane potential levels showed that depolarization and hyperpolarization responses had the same reversal potential of about −70 to −80 mV (Fig. 2). An increase in K^+ ion concentration in the medium from 3.25 to 10 mM led to a shift of the reversal potential toward positive values (Fig. 2a). This is evidence that serotonin induces hyperpolarization and increases membrane conductivity of the neurons by increasing K^+ permeability. However, tetraethylammonium (TEA; 5 mM) and 4-aminopyridine (0.1 mM), which block voltage-dependent K channels, did not affect serotonin-induced hyperpolarization of the neurons (Fig. 2b, c). Consequently, the serotonin-induced increase in potassium conductivity of the membranes is not brought about through the participation of voltage-dependent channels. Evidently Ca-activated K channels, which are insensitive to the action of TEA and of 4-aminopyridine [2], are linked with S_1R.

Since membrane depolarization of the spinal ganglionic neurons mediated by activation of S_2R is accompanied by an increase in input resistance (Fig. 1) and since its reversal potential is close to the potassium equilibrium potential (Fig. 2), it can be tentatively suggested that this reaction is the result of inactivation of channels of the outward K-current, similar to the M-current in neurons of sympathetic ganglia [3].

The rat spinal ganglion can thus be used as a convenient model with which to study central serotoninergic mechanisms, for its neurons respond to serotonin in two ways, which can be separated (for example, in the presence of methylsergide), and which are realized with the participation of different ionic mechanisms: those mediated by S_2R — through reduction of potassium conductivity, and those mediated by S_1AR, by an increase in potassium conductivity of the nerve cell membranes. Hyperpolarization response of spinal ganglionic neurons to serotonin can be used for screening buspirone-like drugs and for searching for selective S_1AR blockers, which do not yet exist.

LITERATURE CITED

1. I. I. Abramets and I. V. Komissarov, Neirofiziologiya, No. 3, 241 (1982).
2. P. G. Kostyuk and O. A. Kryshtal', Mechanisms of Electrical Excitability of the Nerve Cell [in Russian], Moscow (1981).
3. P. R. Adams, D. A. Brown, and A. Constanti, J. Physiol. (London), 330, 537 (1982).
4. C. T. Dourish, P. H. Hutson, and G. Curzon, Trends Pharm. Sci., 7, 212 (1986).
5. H. J. Haighler and G. K. Aghajanjan, Fed. Proc., 36, 2159 (1977).
6. R. S. G. Jones, Neuropharmacology, 21, 511 (1982).
7. D. N. Middlemiss, Trends Pharm. Sci., 7, 52 (1986).
8. S. O. Ogren, K. Fuxe, L. F. Agnati, et al., Neurol. Transm., 46, 85 (1979).
9. S. J. Peroutka and S. H. Synder, Molec. Pharmacol., 16, 687 (1979).
10. R. H. T. Roberts and D. W. Straughan, J. Physiol. (London), 193, 269 (1967).