TABLE 1. Activity of Caffeine and Sydnocarb Separately and in Combination

Parameter studied	Caffeine	Sydnocarb	Caffeine + sydnocarb
	dose, mg/kg		
Conditioned avoidance reflex time of formation (reduction by not less than two-thirds) latent period (shortening by not less than one half)	10	10	5+5
Summation of impulses (facilitation by at least twofold)	10	0,5	5+0,25
Time of swimming (at least twofold increase)	20	10	10+5
Antagonism with hexobarbital (60 mg/kg; shortening of sleep by 50% at $P < 0.001$)	20	10	10+5
Blood pressure, ECG	10	10	5+5
Toxicity D_{50}	250	500	125+250

and sydnocarb can be used with advantage in medical practice. Yet another advantage of the combined use of caffeine and sydnocarb is that they have different mechanisms of action. The former, for instance, according to data in the literature [1-3], increases the cAMP concentration in nerve tissue, by reducing phosphodiesterase activity and causing its degradation, whereas the latter increases adenylate cyclase activity and stimulates its formation.

LITERATURE CITED

1. R. I. Kruglikov, Neurochemical Mechanisms of Learning and Memory [in Russian], Moscow (1981).
2. G. C. Palmer, (editor), Neuropharmacology of Cyclic Nucleotides, Baltimore (1979).
3. N. A. Fedorov, The Biological and Clinical Importance of Cyclic Nucleotides [in Russian], Moscow (1979).

MECHANISM OF THE INHIBITORY EFFECT OF ATYPICAL ANTIDEPRESSANTS AND PSYCHOSTIMULANTS ON SYNAPTOSOMAL MONOAMINE UPTAKE

E. M. Gankina and N. I. Maisov

UDC 615.214.015.4:[612.822.1:577.175.82

KEY WORDS: atypical antidepressants; psychostimulants; monoamine reuptake; synaptosomes.

Antidepressants and psychostimulants can actively inhibit monoamine reuptake into nerve endings in the CNS [3-6, 11], but the character of interaction of different groups of drugs with the hypothetical carriers of neurotransmitters in the presynaptic membrane has not yet been explained. The problem of the presence of three different carriers in the mammalian brain for noradrenalin, dopamine, and serotonin, or a single universal carrier has not yet been settled.

The object of this investigation was to analyze the type of inhibition by atypical antidepressants and psychostimulants of the uptake of noradrenalin, dopamine, and serotonin by the coarse synaptosomal fraction of rat cerebral cortex.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman). Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 52-54, August, 1983. Original article submitted March 3, 1983.

0007-4888/83/9608-1091$07.50

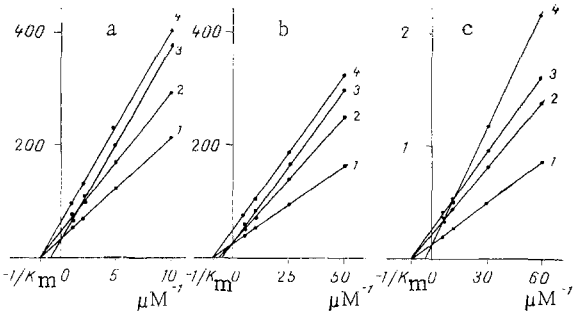

Fig. 1. Effect of imipramine, cocaine, and amphetamine on synaptosomal uptake of noradrenalin (a), dopamine (b), and serotonin (c). a: 1) Control, 2) imipramine (50 μM), 3) amphetamine (1 μM), 4) cocaine (25 μM); b: 1) control, 2) amphetamine (0.5 μM), 3) cocaine (0.5 μM), 4) imipramine (50 μM); c: 1) control, 2) imipramine (1.5 μM), 3) cocaine (0.5 μM), 4) amphetamine (20 μM). Each point represents mean value of 4-6 measurements. Abscissa, μM^{-1}; ordinate: a, b) nmoles/mg protein/min, c) moles/mg protein/min.

TABLE 1. Contstants and Type of Inhibition of Synaptosomal Uptake of Noradrenalin, Dopamine, and Serotonin by Neurotropic Drugs (M ± m)

Drug	K_i, μM		
	noradrenalin	dopamine	serotinin
Noradrenalin	$K_m = 0,67 \pm 0,07$	$0,35 \pm 0,05$	100 ± 10
Dopamine	$0,9 \pm 0,3$	$K_m = 0,12 \pm 0,01$	11 ± 1
Serotonin	12 ± 4	$3,4 \pm 0,6$	$K_m = 0,1 \pm 0,01$
Zimelidine	67 ± 14	61 ± 9	$0,6 \pm 0,1$
Viloxazine	71 ± 15	227 ± 29	54 ± 6
Amphetamine	$1,1 \pm 0,4$	$0,96 \pm 0,2$	$6,5 \pm 1$
Cocaine	20 ± 5*	$0,4 \pm 0,06$	$0,6 \pm 0,1$*
Imipramine	150 ± 30*	55 ± 6*	3 ± 1*

Legend. Asterisk denotes noncompetitive type of inhibition, in all other cases — competitive.

EXPERIMENTAL METHOD

The coarse synaptosomal fraction of cerebral cortex was obtained by the method described previously [1]. For the experiments 50 μl of a suspension of synaptosomes (mean 0.2 mg protein) was taken and added to 1 ml of incubation medium of the following composition: 100 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$ 1.14 mM $MgCl_2$, 5 mM Na_2PO_4, 10 mM glucose, 10 mM sucrose, 0.125 mM pargyline, and 30 mM Tris-HCl, pH 7.4. The incubation medium also contained the drug and the monoamine [^{3}H]dopamine (1.48 GBq/mmole), or [^{3}H]-D,L-noradrenalin (specific radioactivity 629 GBq/mmole), [^{3}H]dopamine (1.48 GBq/mmole), or [^{3}H]serotonin (544 GBq/mmole); all monoamines were from Amersham Corporation, England. Incubation was carried out at 37°C for 3 min with continuous shaking. Binding of the mediator by synaptosomes was stopped by filtering 0.5 ml of incubation medium through membrane filters (Millipore), 25 mm in diameter (pore size 0.45 μ) followed by rinsing with 15 ml incubation medium at 37°C. The washed filters were dried and dissolved in 10 ml scintillation fluid containing 7 ml toluene, 3 ml methyl ester of ethylene glycol, 0.5% 2,5-diphenyloxazole (PPO), and 0.01% bis-[2-(5-phenyloxazolyl)]-benzene (POPOP). Radioactivity was measured with an SL-4000 liquid scintillation counter (Intertechnique, France), with calculation of the mean number of counts per minute. Protein was determined by Lowry's method [7]. The results were subjected to statistical analysis with calculation of mean values and their confidence limits at P = 0.05.

EXPERIMENTAL RESULTS

The results of a study of the effect of the most completely investigated inhibitors of reuptake of noradrenalin, dopamine, and serotonin on the kinetics of synaptosomal transport of these monoamines are given in Fig. 1. It will be clear from Fig. 1a-c, that amphetamine,

structurally similar to monoamines, modified the apparent value of the Michaelis–Menten constant (K_m) for uptake of noradrenalin, dopamine, and serotonin by synaptosomes without affecting the maximal rate of uptake of all the monoamines indicated, evidence of a competitive mechanism of interaction of amphetamine with the corresponding hypothetical carriers of monoamines in the presynaptic membrane. These results confirm the views of Al'tshuler and Granik [3] that amphetamine is complementary to the active center of the hypothetical carrier of noradrenalin, and they also indicate that this psychostimulant is stereochemically complementary to the active centers of two other hypothetical carriers of monoamines. The value of the inhibition constant (K_i) of amphetamine for synaptosomal serotonin uptake, according to our data, is 6.5 μM (Table 1), in agreement with data in the literature [12]. The psychostimulant cocaine inhibited synaptosomal uptake of noradrenalin and serotonin noncompetively. This property of cocaine can be explained by its nonspecific membranotropic action [3]. However, according to our data cocaine competitively inhibited synaptosomal uptake of dopamine (Fig. 1b) but had no effect on uptake of GABA, and it thus exhibited selective affinity for certain sites on the synaptosomal membrane.

Unlike cocaine the antidepressant imipramine noncompetitively inhibited uptake of noradrenalin, dopamine, and serotonin, by synaptosomes (Fig. 1a-c), and also GABA uptake (K_i = 154 μM) [4]. Considering data showing the membranotropic effects of phenothiazine derivatives [4] and their effect on the lipid phase of membranes [2], it can be tentatively suggested that the character of the noncompetitive type of inhibition of reuptake of monoamines by imipramine which was found can be explained by its irreversible membranotropic action.

Values of K_m and K_i and types of inhibition of synaptosomal uptake of monoamines by neurotropic drugs are given in Table 1. Dopamine, as we know can be carried by the noradrenalin transport system in nerve endings and, conversely, noradrenalin can be carried by the dopamine transport system [5, 9], whereas synaptosomal uptake of noradrenalin is competitively inhibited by serotonin [10]. As Table 1 shows, according to our data K_i for serotonin for catecholamine uptake is 10 times higher than the value of K_m, whereas K_i and K_m for catecholamines are of the same order of magnitude, evidence of the greater similarity of the active centers of the catecholamine carriers with each other than between carriers of catecholamines and serotonin. K_i for synaptosomal uptake of noradrenalin by serotonin, according to our observations, was 12 μM, in satisfactory agreement with data obtained on brain slices (K_i = 9 μM for this process) [10]. On the basis of the results it was postulated that the active center of the serotonin carrier is more similar to the center of the dopamine carrier than of the noradrenalin carrier, and also that the active center of the dopamine carrier is more labile in its conformation than centers for other monoamine carriers. Our hypotheses are confirmed by the results obtained with cocaine, which was found to be a competitive inhibitor of uptake for dopamine only (Fig. 1b; Table 1). However, the center of the dopamine carrier evidently has definite limits to its conformational lability, for it is less accessible than the serotonin and noradrenalin centers for viloxazine (Table 1). As regards serotonin uptake, zimelidine proved to be the most active competitive inhibitor (K_i = 0.6 μM), but its possible metabolite norzimelidine, which also is a competitive inhibitor, was 10 times more active than its precursor (K_i = 0.08 μM). According to data obtained by other workers [8] zimelidine also was a specific and highly selective inhibitor of synaptosomal serotonin uptake.

The results thus confirm the view that monoamine transport systems in the mammalian brain are closely interconnected; this is probably the reason why each of the three monoamine neurotransmitters can be carried by all three transport systems.

LITERATURE CITED

1. N. A. Avdulov and N. I. Maisov, Byull. Éksp. Biol. Med., No. 11, 564 (1981).
2. N. A. Avdulov, G. E. Dobretsov, and A. V. Val'dman, Byull. Éksp. Biol. Med., No. 8, 44 (1981).
3. R. A. Al'tshuler and V. G. Granik, Zh. Vses. Khim. Ova. im. D. I. Mendeleeva, No. 2, 171 (1976).
4. N. I. Maisov, "Effect of psychotropic drugs on uptake and release of neurotransmitters in brain synaptosomes," Author's Abstract of Candidate's Dissertation, Moscow (1977).
5. N. I. Maisov and E. M. Gankina, in: Neurochemical Bases of the Psychotropic Effect [in Russian], Moscow (1982)., pp. 66-80.
6. B. K. Koe, J. Pharmacol. Exp. Ther., 139, 649 (1976).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. S. B. Ross, S.-O. Ogren, and A. L. Resyi, Acta Pharmacol. Toxicol., 41, 337 (1977).

9. E. G. Shaskan and S. H. Snyder, J. Pharmacol. Exp. Ther., 175, 404 (1970).
10. K. Sundaran and S. Mahajan, Physiol. Chem. Phys., 13, 523 (1981).
11. D. Wong, J. Horng, and R. Fuller, Biochem. Pharmacol., 22, 311 (1973).

ROLE OF THE HYPOTHALAMIC OPIOID SYSTEM IN MECHANISMS OF MORPHINE ANALGESIA

G. I. D'yachkova and L. V. Kalyuzhnyi

UDC 615.212.7:547.943].015.4:612.826.4.015.2:[547.95:547.943

KEY WORDS: morphine; analgesia; hypothalamus; nalorphine; met-enkephalin.

Descending inhibitory (antinociceptive) mechanisms of the brain stem, in particular, the periventricular structures of the brain — central gray matter, nuclei raphe, and hypothalamus [1], are considered to participate in the pain-relieving action of morphine. However, most workers link the effect of opiates with an action on structures of the mesencephalon and nuclei raphe [10]. It has recently been shown that synthesis of endorphine [8] and enkephalins [6] takes place in the paraventricular region of the hypothalamus, where a high density of opiate receptors [5] and of neurons sensitive to microiontophoretic application of endogenous opiates has been found [4]. In addition, electrical stimulation of this brain region evoked a phenomenon of analgesia [1, 2]. These data indicate a definite role of the paraventricular region of the hypothalamus in the mechanism of the analgesic action of morphine.

The object of this investigation was to compare changes in morphine analgesia during blockade of opiate receptors of the periventricular region of the hypothalamus and in the nuclei raphe by met-enkephalin and nalorphine.

EXPERIMENTAL METHOD

Analgesia was determined in 13 waking rabbits of both sexes, weighing 2.5-3.0 kg, by measuring the latent period (LP) of the withdrawal of the the tail in response to a nociceptive temperature stimulus (the tail-flick test). At the same time the evoked potential (EP) in the sensomotor cortex in response to nociceptive electrodermal stimulation (EDS) of the contralateral hind limb of the animal, evoking a behavioral avoidance response in the unrestrained rabbit, was analyzed. It was shown previously [3] that changes in the amplitude of the second positive wave of the sensomotor cortical EP correlate with changes in pain sensitivity determined by the tail-flick test and with parameters of autonomic responses during injection of morphine and acupuncture stimulation. EP were analyzed on the NTA-1024 amplitude-phase analyzer (Orion), on the basis of 10 realizations. Parameters of EDS were: a single square pulse (1 msec, 9-10 mA). For intracerebral injections, guide cannulas were implanted bilaterally into the cranial bones 3-5 days before the experiment began, in accordance with coordinates from [7], into the paraventricular region of the hypothalamus, and into the region of the large raphe nucleus. Met-enkephalin (100 μg) and nalorphine (100 μg) were injected in a volume of 1 μl by means of a microsyringe in the course of 90 sec. Isotonic NaCl solution in the same volume was injected as the control. The region of injection of the drugs was subsequently examined histologically. Morphine in a dose of 5 mg/kg was injected intravenously.

The numerical data were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Before injection of morphine LP according to the tail-flick test on the rabbits averaged 21.1 ± 7.0 msec (Fig. 1). EP in the sensomotor cortex in response to nociceptive EDS was

Laboratory of Physiology of Pain, P. K. Anokhin Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 54-56, August, 1983. Original article submitted January 31, 1983.

 0007-4888/83/9608-1094$07.50