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ATYPICAL ANTIDEPRESSANTS: EFFECT OF SYNAPTOSOMAL UPTAKE OF SEROTONIN AND GABA

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KEY WORDS: atypical antidepressants; serotonin; GABA; catecholamines.

Ability to inhibit uptake of monoamines by the neuron membrane is a characteristic property of the tricyclic antidepressants [2-4, 7], which also inhibit the uptake of gamma-aminobutyric acid (GABA), although less strongly than serotonin and catecholamines [2, 4]. Besides tricyclic compounds of the imipramine type, another group of preparations has been discovered which, in their chemical structure and spectrum of pharmacological activity, including their effect on neuromediator uptake, differ from the other known antidepressants, but give rise to a definite therapeutic effect in various depressive states [5]. The bicyclic antidepressant trazodone, for instance, like imipramine, selectively inhibits serotonin uptake [11], whereas the original Soviet tetracyclic antidepressant pyrazidol inhibits noradrenalin and GABA uptake only a very little [4].

Since the mechanism of action of the atypical antidepressants has not been adequately studied, it appeared important to compare a number of structurally different antidepressants with respect to their effect on synaptosomal uptake of labeled serotonin and GABA.

EXPERIMENTAL METHOD

The coarse synaptosomal fraction was obtained by centrifugation of a 10% rat brain homogenate in 0.32 M sucrose at 1000g for 10 min. The supernatant was again centrifuged for 20 min at 11,000g. The residues thus obtained, containing synaptosomes, mitochondria, and myelin, were resuspended in 0.7 ml and 0.32 M sucrose per gram weight of original brain. To obtain material for the experiment 50 μl of the resulting suspension of coarse synaptosomal fraction (average 1 mg protein) was added to 1 ml of an incubation medium containing 100 mM NaCl, 6 mM KCl, 2 mM $CaCl_2$, 1.14 mM $MgCl_2$, 5 mM Na_2HPO_4, 10 mM glucose, 100 mM sucrose, 0.125 mM pargidine, and 30 mM Tris-HCl buffer, pH 7.4; the labeled mediator and drugs were added in appropriate concentrations. The concentration of ^{3}H-serotonin in these experiments was 83 nM (specific radioactivity 12 Ci/mmole; from the Radiochemical Centre, Amersham, England), and that of ^{3}H-GABA was 10 μM (specific radioactivity 10 Ci/mmole, from New England Nuclear, USA). Incubation was carried out at 37°C for 20 min with continuous agitation. Binding of mediators was stopped by cooling to 0-5°C. Synaptosomes were isolated from the incubation medium and the quantity of bound mediator determined by our modification [6] of the method of Snyder and Coyle [12]. Radioactivity was measured by means of an SL 4000 (Intertechnique) liquid scintillation counter, and the mean number of counts per minute was calculated. Protein was determined by Lowry's method [9]. The results were subjected to statistical analysis and mean values and confidence limits at the P = 0.05 level calculated.

EXPERIMENTAL RESULTS

In the experiments of series I serotonin and GABA uptake by the coarse synaptosomal fraction depending on substrate

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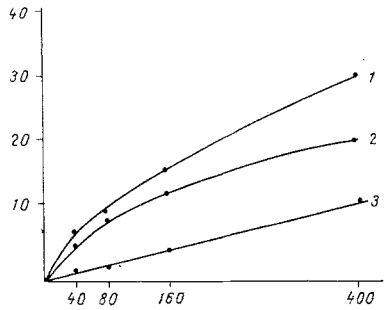

Fig. 1

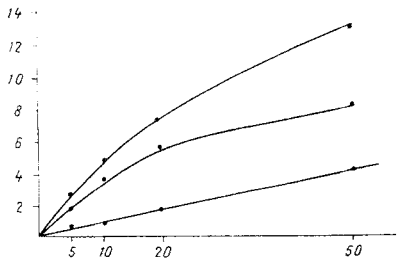

Fig. 2

Fig. 1. Uptake of ^{3}H-serotonin by synaptosomes depending on its concentration in incubation medium. Abscissa, concentration of ^{3}H-serotonin (in nM); ordinate, uptake of ^{3}H-serotonin (in pmoles/mg protein/20 min). 1, 3) Binding of serotonin by synaptosomes at 37 and 0°C; 2) active serotonin uptake at 37°C.

Fig. 2. Uptake of ^{3}H-GABA by synaptosomes depending on its concentration in incubation medium. Abscissa, concentration of ^{3}H-GABA (in μM); ordinate, uptake of ^{3}H-GABA (in nmoles/mg protein/20 min). 1,3) Binding of GABA by synaptosomes at 37 and 0°C, 2) active uptake of GABA at 37°C.

TABLE 1. Effect of Antidepressants with Different Chemical Structure on Uptake (in % of control) of ^{3}H-Serotonin and ^{3}H-GABA by Coarse Synaptosomal Fraction (M ± m)

Substance	^{3}H-Serotonin		^{3}H-GABA	
	concentration of antidepressant, μM			
	50	500	50	500
Control	100±10	100±9	100±10	100±9
Imipramine	31±2	18±5	78±9	14±2
Desmethylimipramine	21±3	19±2	77±9	12±2
Chlorimipramine	26±4	22±3	55±7	4±1
Befuralin	86±10	30±4	121±15	25±5
Trazodone	33±4	22±3	96±10	91±10
Pyrazidol	71±8	26±3	99±15	31±4
Inkazan	35±6	31±6	88±10	49±6
Azaphen	77±8	36±5	70±10	54±6

Legend. 10 nmoles ^{3}H-serotonin and 3.5 μmoles ^{3}H-GABA/mg protein of coarse synaptosomal fraction/20 min at 37°C taken as 100%.

concentration and temperature was determined. It will be clear from Figs. 1 and 2 that at 0°C an increase in the concentration of mediators was accompanied by a linear increase in binding, as a result of nonspecific adsorption of neurotransmitters on the synaptosomes. The radioactivity of the samples at 0°C (adsorption, curves 3 in Figs. 1 and 2) was subtracted from the total radioactivity of the samples at 37°C (curves 1) to give the true curve of active uptake of mediator at 37°C in 20 min (curves 2).

Analysis of these data by the "double reciprocal coordinates" method showed that serotonin and GABA uptake by the coarse synaptosomal fraction is an enzymic process of active transport, for it obeys the Michaelis–Menten kinetic rule. Values of Michaelis constants were 30 μM for GABA and 0.195 μM for serotonin, in satisfactory agreement with results obtained on the pure synaptosomal fraction [2, 6] and with brain slices [8].

On the basis of these results the effect of structurally different antidepressants on serotonin and GABA uptake was investigated in standard concentrations of mediators, namely 10 μM for GABA and 0.08 μM for serotonin, corresponding to concentrations of the mediators in which the velocity of uptake was 0.17 of the maximal.

The results of these experiments (Table 1) showed that all the antidepressants used inhibit serotonin and GABA uptake by synaptosomes to some degree. The most active inhibitors of ^{3}H-serotonin uptake were found to be inkazan and trazodone, in agreement with data on the serotonin-positive action of these drugs [1, 10]. Imipramine and its analogs had a similar or rather stronger inhibitory effect on uptake. In a concentration of 50 μM these antidepressants inhibited ^{3}H-serotonin uptake by 65-70%, but had only a very weak effect on uptake of ^{3}H-GABA. Pyrazidol, azaphen, and befuralin

inhibited serotonin uptake by 60-70%, only in a concentration of 500 μM. All the antidepressants studied in this concentration inhibited GABA uptake by 50-70%. In a concentration of 50 μM only chlorimipramine and azaphen significantly inhibited GABA uptake by 30-45%; all other substances studied did not affect GABA uptake in this concentration.

These data on the inhibitory effect of antidepressants on serotonin and GABA uptake by rat brain synaptosomes are in agreement with analogous results obtained for imipramine in experiments with a pure synaptosomal fraction [2-4].

It can thus be postulated that the inhibitory effect on the neuronal uptake of serotonin plays a definite role in the mechanism of action of the atypical antidepressants inkazan and trazodone, and in this respect they are similar to imiprazine and its analogs. Inhibition of ^{3}H-GABA uptake observed for most substances only in high concentrations, evidently plays a minor role in the effect of the atypical antidepressants.

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EFFECT OF BENACTYZINE AND GALANTHAMINE ON BEHAVIORAL EFFECTS OF APOMORPHINE IN RATS

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KEY WORDS: apomorphine; benactyzine; galanthamine.

Apomorphine stereotypy is one of the most frequently used behavioral tests of the dopaminergic neurotransmitter system. However, analysis of the behavioral effects of apomorphine is often rough and descriptive in character; often other forms of an animal's motor activity are not taken into account although these are important, for example, for analysis of the action of a drug on particular brain structures that participate in the regulation of motor activity and for the interpretation of relations between neurotransmitter systems in these structures.

The object of this investigation was to study the effects of apomorphine depending on the dose used, either separately or in conjunction with the reversible acetylcholinesterase inhibitor galanthamine and the central cholinolytic benactyzine.

EXPERIMENTAL METHOD

Experiments were carried out on 75 noninbred male albino rats weighing 180-250 g. The animals' motor activity was studied by means of an Animex type DSE apparatus, the sensitivity of which was set at the levels of 40 and 10 μA, so that it was possible to record the total number of all movements in a chosen time unit and, at the same time, to analyze motor acts characteristic of locomotor activity. From the total number of all the animal's movements the number of changes of place was subtracted, to give the number of characteristic motor acts of the stereotypy. The experiments were conducted in daylight hours between 9 a.m. and 4 p.m., and the behavior of only one animal was studied each time. The investigation began with a 30-min period of adaptation, and the number of small movements and changes of place during 5 min in

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