response developed slowly, did not reach its initial value, and was often interrupted by periods of marked inhibition of unit activity. Antagonistic interaction between tropane and ACh of this kind was observed in five of seven neurons tested.

It can be concluded from these results that tropane specifically activates muscarinic ACh receptors in the cerebral cortex. While it has low intrinsic activity (effectiveness), by occupying ACh receptors it can enter into competitive relationships with the natural ACh of nerve tissue and reduce the excitatory effect of the latter. The muscarinic cholinolytic effect of atropine is perhaps based on this same property of tropane, which accounts for the greater part of its molecule. Inhibition of activity of some of the cells studied by microiontophoretically applied tropane may be due to its competition with excitatory cholinergic background influences, acting on single neurons from neighboring cortical structures, and also deep brain formations and the periphery.

Excitation of the cortex discovered by the writers previously in response to systemic injection of tropane in doses not exceeding 10 mg/kg was evidently due mainly to activation of adrenoreceptors of subcortico-cortical excitatory pathways [1]. After systemic injection of tropane in larger doses, more than 20 mg/kg, we observed inhibition of electrical activity of cerebral cortical neurons. The cause of this inhibition may be the cholinolytic action of tropane described above. This same property of tropane may be responsible for the inhibition of central nervous processes which develop in the case of overdosage with its derivatives, such as cocaine, atropine, and so on.

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EFFECT OF ATYPICAL NEUROLEPTICS CARBIDINE AND SULPIRIDE ON STRIATAL SYNAPTOSOMAL TYROSINE HYDROXYLASE ACTIVITY IN THE RAT BRAIN

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Neuroleptics, both typical and atypical, have the property of accelerating dopamine (DA) turnover in the brain, the limiting factor in its biosynthesis being tyrosine hydroxylase (TH) [4]. It is suggested that the point of application of atypical neuroleptics may be presynaptic dopamine receptors (autoreceptors), located on terminals of dopaminergic neurons and participating in regulation of the biosynthesis and release of the neurotransmitter [13].

The aim of this investigation was to study the effect of the original Soviet neuroleptic carbidine* and to compare it with that of sulpiride on regulation of TH activity in synaptosomes of the rat corpus striatum. Sulpiride (a benzamide derivative) is one of the best known atypical neuroleptics [7]. Carbidine is a derivative of γ-carboline and has a unique spectrum of action, combining features of a neuroleptic and an antidepressant [1]. The neurochemical mechanisms lying at the basis of the psychotropic action of carbidine have received little study. Only indirect evidence has been obtained that the drug can modify DA biosynthesis [2, 3].

*3,6-dimethyl-1,2,3,4,4a,9a-hexahydro-γ-carboline di-HCl.

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TABLE 1. Effect of Carbidine and Sulpiride on TH Activity and Its Inhibition by DA during Incubation of Synaptosomes in Buffer Containing 5 mM K^+ (data given in %)

Preparation and its concentration, M	Buffer	Buffer + DA (10^{-6} M)	Buffer + nomifensine (10^{-5} M) + DA (10^{-6} M)	Buffer + nomifensine (10^{-5} M)
A. Control	100±4 (35)	53±2 (33)	66±4[a**] (31)	110±5 (25)
Carbidine				
10^{-5}	104±5 (23)	56±2 (10)	—	—
10^{-6}	105±4 (25)	52±3 (35)	63±4 (33)	106±7 (24)
10^{-7}	101±3 (42)	56±3 (19)	—	—
10^{-8}	100±3 (40)	56±2 (19)	—	—
B. Control	100±4 (30)	52±2 (20)	61±3[a*] (23)	110±5 (25)
Sulpiride				
10^{-5}	105±3 (19)	59±2[a*] (25)	74±4[b*] (24)	113±5 (25)
10^{-6}	107±3 (20)	57±2[a*] (19)	—	—
10^{-7}	103±3 (20)	—	—	—
10^{-8}	101±3 (20)	—	—	—

Legend. Enzyme activity in picomoles ^3H_2O/mg protein/min in variant A (control) is 3.2 ± 0.2, in variant B (control) 2.6 ± 0.1. a) Difference compared with DA (10^{-6} M) in control, b) difference compared with DA (10^{-6} M) + nomifensine (10^{-5} M) in control. *P < 0.05, **P < 0.01. Here and in Table 2, number of measurements shown in parentheses.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-220 g. The rats were decapitated, the brain removed, and the corpus striatum isolated. In one experiment corpora striata from four rats were pooled. TH activity was determined by the method in [5] with some modifications. The brain tissue was placed on ice, weighed, and homogenized in cold 0.32 M sucrose solution in a volume of 1:10. The homogenate was centrifuged for 10 min at 1000 g, after which the supernatant was centrifuged for 25 min at 20,000 g. The residue of P_2 was twice washed with Krebs' phosphate buffer of the following composition (in mM): NaCl 124, KCl 5, Na_2HPO_4 20, $MgCl_2$ 1.3, KH_2PO_4 1.2, glucose 10, $CaCl_2$ 0.75, and homogenized in the same medium in the ratio of 1:10 (calculated per wet weight of tissue). The samples were incubated with purified [^{3}H]tyrosine (L-tyrosine-3,5-$^3H_2$, Hungary) on a water bath at 37°C for 15 min. The composition of the incubation medium was: 50 μl of NSD-1015, $4 \cdot 10^{-3}$ M (an inhibitor of decarboxylase of aromatic L-amino acids), 50 μl of Krebs' phosphate buffer, or solution of the test substance in buffer, 50 μl of the suspension of synaptosomes, and 50 μl of tyrosine, $8 \cdot 10^{-6}$ M (the quantity of label per sample was about 10^6 cpm). The reaction was stopped by addition of 400 μl of 3.5% TCA solution. After centrifugation for 5 min at 5000g, 0.5 ml of the supernatant was applied to column measuring 0.5 × 2 cm, filled with Dowex 50W × 4 (H^+) resin, 100-200 mesh. The tritiated water formed during the reaction was separated on columns and collected in flasks with Bray's scintillator (7 ml). The column were washed twice with 0.5 ml distilled water, which also was collected in the flasks. Radioactivity was measured with an Intertechnique SL-4221 liquid β-counter. The results were subjected to statistical analysis by the t and U tests and expressed as percentages of the control. "Blind" samples were incubated at 0°C. Protein was determined by Lowry's method [6].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that both neuroleptics (carbidine and sulpiride) had no appreciable effect on TH activity of synaptosomes incubated in buffer with a normal K^+ content. When a buffer with an increased K^+ concentration (30 mM) was used, inducing depolarization of the synaptosomes, sulpiride did not change TH activity, but carbidine, in concentrations of 10^{-5} and 10^{-6} M, reduced it moderately (Table 2). In a concentration of 10^{-6} M, DA caused a definite decrease (almost by half) in the rate of hydroxylation of tyrosine in the synaptosomes, in agreement with data in the literature [8]. Carbidine had no effect on the inhibitory action of DA on TH activity under normal buffer conditions but definitely potentiated it in buffer with a raised K^+ concentration. By contrast to this, sulpiride significantly depressed the inhibitory action of DA on TH activity both in buffer containing 5 mM K^+ and in buffer with a raised K^+ concentration (Tables 1 and 2); this effect, moreover, was manifested about equally when the neuroleptic was used in concentrations of 10^{-5} and 10^{-6} M. A similar but stronger effect of abolition of the inhibitory influence of DA on the rate of hydroxylation of tyorosine in the synaptosomes was found in experiments in which DA uptake by synaptosomes was inhibited by nomifensine (10^{-5} M). If Krebs' phosphate buffer with 5 mM K^+ was used nomifensine weakened the action of DA whether carbidine was

TABLE 2. Effect of Carbidine and Sulpiride on TH Activity and Its Inhibition by DA during Incubation of Synaptosomes in Buffer Containing 30 mM K^+ (data given in %)

Preparation and its concentration, M	Buffer	Buffer + DA (10^{-6} M)	Buffer + nomifensine (10^{-5} M) + DA (10^{-6} M)	Buffer + nomifensine (10^{-5} M)
A. Control	100±6 (30)	53±5 (23)	73±4[b***] (28)	114±3[a**] (15)
Carbidine				
10^{-5}	92±2[a*] (10)	60±2 (8)	—	—
10^{-6}	91±2[a*] (10)	46±2 (23)	61±4[c*] (22)	105±2[d*] (14)
10^{-7}	102±4 (30)	58±2 (18)	—	—
10^{-8}	104±4 (23)	52±4 (18)	—	—
B. Control	100±6 (15)	44±2 (15)	62±2[b***] (13)	114±3[a***] (15)
Sulpiride				
10^{-5}	107±4 (15)	53±2[b***] (15)	74±4[c**] (15)	116±2 (14)
10^{-6}	108±3 (15)	52±3 (15)	—	—
10^{-7}	106±3 (15)	—	—	—
10^{-8}	104±4 (29)	—	—	—

Legend. Enzyme activity in picomoles ^3H_2O/mg protein/min in variants A and B (controls) 3.0 ± 0.2. a) Difference compared with control, b) difference compared with DA (10^{-6} M) in control, c) difference compared with nomifensine (10^{-5} M) + DA (10^{-6} M) in control, d) difference compared with nomifensine (10^{-5} M) in control. *P < 0.05, **P < 0.01, ***P < 0.001.

present or not (Table 1). If nomifensine was added to medium with an increased potassium concentration, the inhibitory effect of DA was considerably reduced compared with the control. Carbidine in this case had the opposite action, i.e., it potentiated inhibition caused by addition of DA in the standard concentration of 10^{-6} M. In the presence of nomifensine, both in buffer containing 3 mM K^+ and in buffer with 30 mM K^+, sulpiride definitely reduced the inhibitory effect of DA on synaptosomal TH activity (Tables 1 and 2).

Inhibition of TH activity can take place both by activation of postsynaptic receptors of DA by its agonists [8-10] and through the action of the transmitter on so-called autoreceptors, located presynaptically [13, 14]. According to another point of view, however, the inhibitory action of DA on TH activity is effected without the participation of a receptor mechanism, by intracellular inhibition of the enzyme by DA, either assimilated by the presynaptic terminal or newly synthesized [11, 12]. In this investigation nomifensine, an inhibitor of DA uptake, which appreciably weakens the effect of DA [11], was used. Conversion of dopa formed in the course of the reaction into endogenous DA was prevented in these experiments by addition of the dopadecarboxylase inhibitor NSD-1015 to the incubation mixture. Under these conditions, sulpiride significantly weakened the inhibitory effect of DA. Nomifensine itself had a directly opposite action: The inhibitory effect of DA was considerably reduced both when buffer with 5 mM K^+ or buffer with a raised potassium concentration was used. This may indicate that TH activity is regulated in two ways: by intracellular inhibition by DA and by the action of DA or its agonist through presynaptic autoreceptors. The effect of sulpiride can be explained in this case as the result of inhibition of the latter. The other neuroleptic, carbidine, had an effect diametrically opposite to that observed in the case of sulpiride. Reduction of TH activity by carbidine in buffer with K^+ in a concentration of 30 mM can be explained on the grounds that carbidine, in the presence of depolarization of the neuronal membrane, largely penetrates into synaptosomes and can interact directly with TH. From this same point of view we can explain the effects of carbidine observed in experiments with K^+-stimulated synaptosomes in the case of addition of DA or DA and nomifensine. It can be postulated that presynaptic autoreceptors of DA are pharmacologically similar to type D_2 receptors, for we know that sulpiride, a D_2-receptor antagonist, weakens the inhibitory action of their agonist lysuride on TH activity [15].

It can thus be concluded from these results that significant differences exist in the mechanism of action of carbidine and sulpiride. Carbidine can exert a definite inhibitory action on TH activity; this effect, moreover, is manifested only on depolarization of the synaptosomes and is unconnected with any involvement of DA autoreceptors. These observations agree with those published previously [3]. The action of sulpiride is independent of a change in the K^+ concentration in the medium, and it is evidently mediated through presynaptic DA autoreceptors. In experiments in vitro on rat striatal synaptosomes carbidine and sulpiride had opposite effects on the rate of tyrosine hydroxylation: Sulpiride depressed the regulatory inhibitory effect of DA whereas carbidine potentiated it.

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EFFECT OF A TETRAPEPTIDAMIDE NITRO ANALOG AND OF MORPHINE ON THE CEREBRAL CIRCULATION

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Enkephalins perform the function of neurotransmitters or modulators in the CNS. Their interaction with mediator systems of the brain has been demonstrated. It has also been found that central adrenergic and GABA-ergic mechanisms participate both in regulation of the cerebral circulation [3, 5, 6] and in realization of the analgesic effect of morphine [10]. It is therefore important to study the effect of a synthetic analog of the enkephalins, namely a tetrapeptidamide nitro analog, which has an analgesic action on the cerebral circulation, and to compare its effects with those of morphine. No information on the effect of enkephalins and their analog on the cerebral circulation could be found in the literature. As regards morphine, according to observations made by most investigators, the drug increases the cerebral blood flow and lowers the tone of the cerebral vessels [2, 7-9].

The investigation described below was devoted to a study of the effect of the tetrapeptidamide nitro analog (TNA), compared with that of morphine, on the cerebral circulation and its nervous control. The cerebrovascular effects of these compounds were studied also after blockade of GABA-receptors by bicuculline.

EXPERIMENTAL METHOD

Experiments were carried out on 69 cats weighing 3-4 kg under general anesthesia (urethane, chloralose) with artificial ventillation of the lungs.

The flow of blood into the brain through the carotid artery was determined, after careful ligation of the extracranial branches by means of an electromagnetic flowmeter (Nihon Kohden). The EEG from the parietal region, the ECG in lead II, and the blood pressure in the femoral artery (BP) were recorded simultaneously. Tonic activity and reflex discharges were recorded in the sympathetic nerves of the renal plexus [1]. The vascular component of the action of the substances on the cerebral hemodynamics was differentiated by separate

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