# DIFFERENT EFFECTS OF TYPICAL AND ATYPICAL NEUROLEPTICS ON $K^+$-STIMULATED DOPAMINE RELEASE FROM ISOLATED RAT STRIATUM

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Blockade of pre- and postsynaptic dopamine (DA) receptors by a single injection of neuroleptics leads to an increase in the spike discharge of neurons belonging to the nigrostriatal system of the brain and associated neurochemical changes, in particular, acceleration of biosynthesis and metabolism and increased release of DA from the terminals [10, 12]. It has been suggested that increased DA release in the striatum under the influence of neuroleptics can make a definite contribution to the formation of the atypical pharmacologic profile of these substances due to competition between the released mediator and neuroleptics at the level of postsynaptic DA receptors [3, 10]. It is generally considered that the cause of the increased DA release in the striatum under the influence of neuroleptics is blockade of terminal autoreceptors belonging to the $D_2$ type [12, 15]. However, there is also evidence in support of a possible role of type $D_1$ dopaminergic receptors in the regulation of this process [6]. Increased release of DA during the action of neuroleptics has been demonstrated in many investigations both in vivo and in vitro [2, 3, 7, 10-12, 15]. At the same time, during the study of haloperidol, chlorpromazine, and other neuroleptics, either a decrease in DA release from striatal slices or no effect was observed [7, 11, 14].

The aim of this investigation was to study the effect of different types of neuroleptics on $K^+$-stimulated release of endogenous DA from the isolated rat striatum.

## EXPERIMENTAL METHOD

To study the relative role of dopamine $D_1$ and $D_2$ receptors in the regulation of DA release, selective antagonists of these receptors SCH 23390 and raclopride were used. Experiments were carried out on male Wistar rats weighing 280-320 g. After decapitation, the striatum (80-90 mg) was isolated from both cerebral hemispheres in the cold. Each striatum was cut transversely and placed in carbogenized buffer of the following composition (in mM): NaCl 111, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.64, $NaHCO_3$ 25, $KH_2PO_4$ 1.2, $Na_2EDTA$ 0.054, ascorbic acid 0.28. The striatum from each animal was investigated in a separate thermostated chamber at 37°C. The first incubation lasted 1 h, after which the buffer was replaced by fresh with the addition of the substance in the test concentration. After 10 min the buffer was drawn off and this was followed by a second incubation for 10 min with the same substance, but in a medium with an increased $K^+$ concentration (28 mM) and an equimolarly reduced $Na^+$ concentration. Both 10-min samples, reflecting basal and $K^+$-stimulated DA release, after precipitation on alumina were used to determine DA by HPLC with electrochemical detection. If DA was precipitated on previously activated alumina belonging to the "Katekholkhrom" kit (MNPP "DIA-M," Moscow) in the presence of 1 M Tris buffer (pH 8.6). After the sample had been shaken for 10 min and the alumina washed three times with deionized water, the precipitated DA was

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TABLE 1. Effect of Substances on $K^+$-Stimulated Dopamine Release from Isolated Striatum

| Substance | Concentration, M | Number of specimens | Per cent of stimulated DA release |
|---|---|---|---|
| Control | — | 25 | 100±5 |
| Haloperidol | $10^{-7}$ | 5 | 108±28 |
| Haloperidol | $10^{-6}$ | 9 | 91±9 |
| Haloperidol | $10^{-5}$ | 5 | 69±14* |
| Trifluoperazine | $10^{-6}$ | 6 | 86±16 |
| Metoclopramide | $10^{-6}$ | 5 | 104±38 |
| Tiapride | $10^{-6}$ | 10 | 78±16 |
| Sulpiride | $10^{-6}$ | 5 | 219±44* |
| Thioridazine | $10^{-6}$ | 4 | 137±7* |
| Clozapine | $10^{-4}$ | 4 | 141±16* |
| Cis-carbidine | $10^{-6}$ | 5 | 151±16* |
| Trans-carbidine | $10^{-6}$ | 7 | 155±17* |
| Remoxipride | $10^{-6}$ | 5 | 148±16* |
| Raclopride | $10^{-6}$ | 10 | 160±16* |
| SCH 23390 | $10^{-6}$ | 5 | 105±13 |
| Raclopride + SCH 23390 | $10^{-6}$ + $10^{-6}$ | 5 | 148±22* |

**Legend.** Data given as ratio, in per cent, between stimulated DA release (232 ± 12 pmoles/min/mg tissue) and basal (58 ± 7 pmoles/min/mg tissue) value in control specimens (mean ± standard error of the mean). Asterisk indicates significance of differences from control at $p < 0.05$ level (Student's t test).

eluted with 0.1 M $HClO_4$. The samples were analyzed on a reverse-phase column (3 × 150 mm, $C_{18}$, 5 $\mu$, MNPP "Élsiko," Moscow), using 0.1 M citrate-phosphate buffer, containing 0.25 mM sodium octanesulfonate, 0.1 mM EDTA, and 8.5% acetonitrile (pH 3.6). Detection was carried out on a glass-carbon electrode at +0.8 V (LC-4B, "BAS," USA). The following substances were used in the work: haloperidol (from "Janssen Pharmaceutical," Belgrium), trifluoperazine (USSR), metoclopramide ("Arzneimittelwerk," Germany), tiapride ("SESIIF," France), sulpiride ("Serva," Germany), thioridazine ("Polfa," Poland), clozapine ("Arzneimittelwerk," Germany), remoxipride ("Astra," Sweden), raclopride ("Astra," Sweden), SCH 23390 ("Schering," USA), and *cis*- and *trans*-carbidine (USSR). $K^+$-depolarization leads to an increase of 400% in the DA concentration in the perfusion fluid. Effects of the substances against the background of $K^+$-depolarization were assessed by comparison with the above-mentioned values, using Student's t test.

## EXPERIMENTAL RESULTS

The effects of the substances on $K^+$-stimulated DA release from the isolated striatum are given in Table 1. Haloperidol, when added in concentrations of $10^{-7}$ and $10^{-6}$ M, did not change the $K^+$-stimulated DA release from the striatum. With an increase in concentration to $10^{-5}$ M a statistically significant decrease in DA release was observed. In a concentration of $10^{-6}$ M trifluoperazine, metoclopramide, and tiapride had no effect on stimulated DA release, whereas sulpiride, thioridazine, clozapine, remoxipride, and raclopride, isomers of carbazine, raised its level considerably. Compound SCH 23390 ($10^{-6}$ M) had no significant effect on the DA concentration in the perfusion fluids during $K^+$-stimulation, in exactly the same way as elevation of the level of stimulated DA release induced by raclopride ($10^{-6}$ M). Incidentally, none of the substances studied caused any significant changes in the level of basal DA release from the isolated striatum (results not given).

Neuroleptics, if administered once only, raise the extracellular DA level in the striatum of unrestrained rats [3, 12, 15]. This effect is regarded as the result of blockade by the neuroleptics of terminal dopamine autoreceptors [10, 12]. However, several workers who studied DA release in vitro found no increase in its level in the striatum. For instance, a decrease in electrically and $K^+$-stimulated release of labeled and endogenous DA from striatal slices, was found with haloperidol in concentrations over $10^{-6}$ M [7, 11, 14]. Chlorpromazine also had a similar action [11, 14]. Incidentally, haloperidol, trifluoperazine, metoclopramide, and tiapride, like chlorpromazine, are cataleptogenic neuroleptics [13], whereas sulpiride, thioridazine, clozapine, remoxipride, and raclopride and the carbidine isomers can be classed in the group of noncataleptogenic (atypical) neuroleptics [3, 8, 13]. Separation of the typical and atypical neuroleptics in this way, based on their effect on DA release in the striatum, was demonstrated previously in experiments in vivo to study the effects of chronic neuroleptic administration [1]. It was shown that haloperidol, chlorpromazine, and metoclopramide, unlike clozapine, sulpiride, and thioridazine, reduce DA release in the striatum under these conditions. This effect must be regarded as the result of depolarization inactivation of dopaminergic neurons, which can take place at the level both of the body and of the axon of the neuron [1, 4, 5]. It is suggested that the cause of the development of a depolarization block during chronic neuroleptic administration may be the long-term blocking of postsynaptic DA receptors, inducing hyperactivation of neurons of the substantia nigra by a mechanism of feedback through striatonigral pathways. There are, however, grounds for supposing that this mechanism of origin of depolarization inactivation and subsequent reduction of DA release is not the only one. It has been shown, for instance, that haloperidol can induce a depolarization block of nigrostriatal neurons by a single injection into rats partially denervated by 6-hydroxydopamine [4]. It is suggested that increased sensitivity of neurons to depolarization inactivation in this case may be the result of changes at the membrane level caused by the toxic agent. Apomorphine, moreover, did not change the fall of the extracellular DA level in the striatum induced by chronic haloperidol administration, whereas the hyperpolarizing effect of apomorphine, counteracting the depolarization block of nigrostriatal neurons, is well known [5]. The possibility that the effects observed may arise through accumulation of neuroleptics in neuron membranes during their long-term exposure likewise cannot be ruled out [2, 14].

In the light of the facts described above, the effects of typical neuroleptics observed in the present investigation can logically be regarded as the result of the nonreceptor influence of the substances, masking possible enhancement of DA release due to dopamine autoreceptor blockade. This conclusion is in agreement with data showing the absence of an effect of apomorphine on the haloperidol-induced reduction of electrically stimulated DA release from striatal slices [14]. The following mechanisms lying at the basis of the membranotropic action of neuroleptics can be suggested: a local anesthetic effect, interaction with $Ca^{2+}$-dependent processes, involvement of local neuronal chains in the isolated striatum, maintaining functioning of the feedback mechanism at the striatal level between postsynaptic blockade of DA receptors and the level of activity of presynaptic terminals [2, 11]. A possible contribution of $K^+$ depolarization (28 mM), which we used to stimulate DA release, likewise cannot be ruled out.

It remains unclear which of these possible mechanisms holds the key. It is important to note that the conditions of the present investigation (10 min of $K^+$-depolarization, 20 min of perfusion of the isolated striatum in the presence of the neuroleptic, and micromolar concentrations of the substances) enabled a distinction to be drawn between typical and atypical neuroleptics as regards their effect on stimulated DA release. High activity of the neuroleptics in relation to DA release in the striatum, which was demonstrated previously in experiments both in vivo [3] and in vitro, in this investigation can be regarded as a mechanism determining the atypical properties of the substance. It can be suggested that the released DA can compete with molecules of the neuroleptic for postsynaptic receptors, thereby counteracting the postsynaptic receptor blockade which lies at the basis of development of extrapyramidal disorders [3, 10]. Incidentally, the concentrations of the neuroleptics used in this study were close to their therapeutic level, capable of being reached in the human brain during treatment of patients [9].

The fact that the dopamine $D_1$ receptor antagonist SCH 23390 had no effect on $K^+$-stimulated DA release, like the effect of enhancement of this process under the influence of raclopride, a type $D_2$ antagonist, confirmed the dominant role of terminal autoreceptors of $D_2$ type in the regulation of DA release in the striatum [12, 15].

Typical neuroleptics thus do not affect $K^+$-stimulated DA release from the isolated striatum, or depress it, whereas atypical neuroleptics lead to a marked rise of its level. DA release in the striatum is controlled through dopamine receptors of the $D_2$ type and is independent of the degree of type $D_1$ receptor blockade.

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# EFFECT OF CEREBROCRAST ON LOCAL CEREBRAL BLOOD FLOW AND EEG IN CATS AFTER BRAIN HEMORRHAGE

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**KEY WORDS:** brain hemorrhage; calcium antagonists; cerebral blood flow; EEG

The high efficacy of 1,4-dihydropyridine derivatives in the treatment of acute cerebrovascular disturbances [7, 13] justifies a further search for correctors of the cerebral hemodynamics among this group of compounds. A new preparation, cerebrocrast has been synthesized at the Institute of Organic Synthesis, Academy of Sciences of Latvia, and has a marked selective action on the cerebral vessels and significantly increases the volume velocity of the total cerebral blood flow in anesthetized animals [5].

The aim of this investigation was to study the effect of cerebrocrast on the local cerebral blood flow of conscious animals, under normal conditions and with chronic vasospasm after intracerebral hemorrhage, and to compare it with the effect of the most active cerebral vasodilator of the 1,4-dihydropyridine group, namely nimodipine, and also to evaluate the effect of cerebrocrast on the EEG in this pathology.

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