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MEDIATION BY THE CORTICOSTRIATAL INPUT OF THE IN VIVO INCREASE IN RAT STRIATAL ACETYLCHOLINE CONTENT INDUCED BY 2-CHLOROADENOSINE

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Electrophysiological (1) and biochemical (2,3) studies have given evidence for the existence of a corticostriatal pathway in which glutamate may be the excitatory neurotransmitter. This pathway appears to innervate cholinergic neurons intrinsic to the striatum and to regulate their activity as the following results indicate: a), cholinergic interneurons are destroyed by the intrastriatal application of the neurotoxin, kainic acid, a conformationally restricted analog of glutamic acid and b), cholinergic neurotransmission is compromised after long-term decortication, i.e., the acetylcholine turnover rate is decreased (4), the sodium-dependent high affinity uptake of choline is reduced (5) and the muscarinic action of oxotremorine in increasing striatal acetylcholine content is prevented (Ladinsky et al., unpublished result).

Research outlined in the present communication demostrates that the corticostriatal pathway mediates the depressant effect exerted by 2-chloroadenosine on striatal cholinergic interneurons. This result is suggestive of an indirect action of the putative adenosine receptor agonist on striatal cholinergic neurotransmission.

Female CD-COBS rats (Charles River, Italy), body weight 175-200 g were used. The rats were killed by fast focussed microwave irradiation to the head (1.3 KW at 2.45 GHz for about 4 sec.) using an adapted commercial microwave oven (Medical Engineering Consultants, Peabody, Ma., U.S.A.). The brain was quickly removed and the striatum dissected. Acetylcholine and choline were measured by the radioenzymatic method of Saelens et al. (6) with modifications by Ladinsky et al. (7). Choline o-acetyltransferase activity was measured by the method of Fonnum (8). Striatal noradrenaline, dopamine and serotonin contents were measured by electrochemical detection coupled with high performance liquid chromatography (9,10).

Frontal decortication was performed in anaesthetized (diethylether) rats as follows: the animals were positioned in a stereotaxic apparatus and the skull was opened laterally to bregma, 6 mm along the frontotemporal suture, 2 mm depth. A horizontal cut of the right hemisphere was made by a glass knife fashioned from a cover slip. In sham-operated animals, the skull was opened but no lesion made. The experiment was performed 14 days after the lesion. In this condition, the uptake of (^{3}H)glutamic acid (Amersham International Ltd., U.K.), as estimated by the method of Divac et al. (2), was decreased by 55% in the striata ipsilateral to the

lesion as compared to striata removed from sham-operated rats (from 5.73±1.7 to 2.63±0.13 nmoles glutamic acid/min/mg protein (means and S.E.M., n=6; $p < 0.01$), indicating the extensive impairment of corticostriatal glutamatergic input.

The contents of noradrenaline (102.2±6.4 ng/g), serotonin (166.5±9.8 ng/g), dopamine (10.7±0.4 µg/g) and choline (23.0±1.0 nmoles/g) as well as the activity of choline acetyltransferase (1.9 nmoles/min/mg protein) in the striatum were not altered.

2-Chloroadenosine (Sigma, St. Louis, Mo. U.S.A.) was dissolved in sterile saline and was administered intracerebroventricularly in a volume of 5 µl to alert animals through a Portex PP 10 polyethylene guide cannula implanted in the third ventricle 48h earlier.

The time course showed that the drug, at the peak dose of 20 µg, significantly increased the level of striatal ACh within 15 min of its administration. The maximal increase of approximately 20% was attained at 30 min and the effect of the drug was terminated by 60 min (Fig. 1). The ACh-increasing property is consistent with the inhibition of (^{3}H)acetylcholine release from striatal slices provoked by adenosine (11). Pretreatment with the adenosine antagonist, theophylline (120 mg/kg, i.p., 15 min before), completely antagonized the cholinergic effect of 2-chloroadenosine suggesting that an activation of a purinergic receptor is operative in the action of the drug (Table 1). In a similar dose range, theophylline was proved to antagonize the

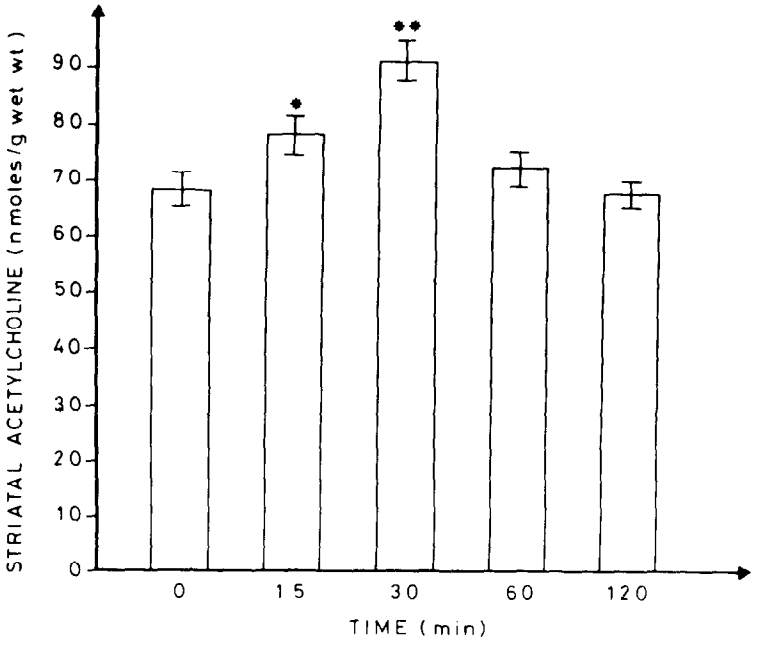

Fig. 1. Time course of the effect of 2-chloroadenosine on rat striatal acetylcholine content The drug was dissolved in sterile saline solution; 20 µg were injected intracerebroventricularly in a 5 µl volume through a polyethylene cannula implanted 48 h before the experiment. * = $p < 0.05$ and ** = $p < 0.01$ as determined by Dunnett's test. The data are the means ± S.E.M. (vertical bars) of 5 rats per group.

Table 1. The effect of theophylline or frontal decortication on the 2-chloroadenosine-induced increase in striatal acetylcholine content.

Treatment in columns C & D	A Vehicle or Sham	B 2-chloro-adenosine	C Treatment Alone	D Treatment + 2-chloroadenosine
Theophylline	68.2 ± 1.1	* 79.0 ± 1.9	68.5 ± 1.9	67.0 ± 2.2
Frontal decortication	65.1 ± 2.1	* 78.2 ± 1.3	65.3 ± 2.6	66.8 ± 3.2

Theophylline was administered at the dose of 120 mg/kg, i.p. and the rats were killed after 45 min. The drug caused slight hypermotility.
2-Chloroadenosine was administered i.c.v. at a dose of 20 µg/5µl and the animals were killed after 30 min. This treatment caused sedation which was not mitigated either by theophylline pretreatment or by the unilateral frontal decortication.
The data show the means and S.E.M. (n=7).
* $p < 0.01$ vs the vehicle or sham group; ANOVA (2x2) test.

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depressant effect of iontophoretically applied adenosine on the spontaneous firing rate of cortical neurons (12). By itself, theophylline did not alter striatal acetylcholine content.

After chronic unilateral decortication, the increase in actylcholine content induced by 2-chloroadenosine was completely prevented. Acetylcholine content was not altered by the lesion itself. It is thus clear that 2-chloroadenosine's cholinergic action is dependent upon an integral corticostriatal pathway. From these data it can be inferred that the drug, through an activation of purinergic receptors, reduces the release of an excitatory neurotransmitter, probably glutamate, from the corticostriatal nerve terminals. This results in a depression of the cholinergic interneurons, as revealed by the increase in the content of acetylcholine subsequent to a reduced release of this neurotransmitter.

Decortication, like 2-chloroadenosine, should have produced an increase in acetylcholine content as a result of the removal of the tonic positive input. It is unclear at present why this did not occur (13), but it is reasonable to assume that a compensatory mechanism ensued post-lesion to return the acetylcholine content to normal. This is substantiated by the finding that the turnover rate of acetylcholine is decreased after chronic decortication (4).

The present results provide the first evidence that the depression of synaptic transmission exerted by the purinergic agonist, 2-chloroadenosine, is indirect, at least as regards the interneuronal cholinergic system in the striatum. Further work is in progress to gain a deeper insight into the mechanisms of this phenomenon.

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