Role of striatal dopamine receptors in the regulation of cerebellar cyclic GMP

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It is now accepted that 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) function in synaptic transmission as putative second messengers [1-3]. The cAMP and cGMP content of a given brain nucleus shifts in conditions that cause either a persistent stimulation or a blockade of postsynaptic receptors regulating guanylate or adenylyl cyclase activity [4-6]. Thus, by measuring the cAMP or cGMP content of brain nuclei of rats killed with a microwave beam focused to the skull, one can assess activity changes in the synaptic input to a given brain nucleus even when the transmitter involved is unknown.

Cerebellar cortex coordinates body movements by regulating the amount of gamma-aminobutyric acid (GABA) which is released from Purkinje cell terminals at synapses in deep cerebellar nuclei (Fig. 1) [7, 8]. The overall function of cerebellum is to transduce the excitatory input reaching this structure through mossy and climbing fibers into an inhibitory signal to the cells of deep cerebellar nuclei [9].

One of us has shown that the cGMP content of the deep cerebellar nuclei is decreased when GABA receptors are specifically activated and is increased when GABA receptor function is decreased [10]. Hence, by measuring the cGMP content of deep cerebellar nuclei, one can make inferences on the release rate of GABA from Purkinje cell terminals.

Although we have precise information on the fine morphology of the synaptic contacts formed by climbing or mossy fibers and the various types of cerebellar neurons [7], the molecular nature of the transmitter released from mossy, parallel or climbing fibers (Fig. 1) is unknown.

Fig. 1 shows that mossy fibers innervate granule cells and neurons of deep cerebellar nuclei; these neurons are also innervated by climbing fibers and Purkinje cell axons, but not by the axon terminals of granule cells. The axons of the granular cells form the parallel fibers which represent the most abundant excitatory input to basket, Purkinje and Golgi cells.

The cells which are depicted in black in Fig. 1 secrete the inhibitory transmitter GABA [8], in contrast, climbing, mossy and parallel fibers secrete an unknown excitatory transmitter. Mossy fibers excite deep cerebellar nuclei and by activating parallel fibers (Fig. 1) cause a diffuse stimulation of Purkinje cells [9]; in fact a single parallel fiber makes synaptic contacts with a great number of Purkinje cells. Though the final result of the mossy fiber activation is an excitation of deep cerebellar nuclei and Purkinje cells, the direct connections of parallel fibers with Golgi cells (Fig. 1) makes it difficult to predict whether a given activation of mossy fibers is transduced into Purkinje cells excitation. In contrast to the parallel fibers, each climbing fiber produces a focused stimulation of a few Purkinje, basket and Golgi cells. The simultaneous stimulation of basket and Golgi cells creates a feed-forward inhibition (Fig. 1) which results in a typical pattern of Purkinje cell discharge known as climbing fiber discharge [9].

Recent evidence suggests that the caudate nucleus is the major source of excitatory afferents to the cerebellum and that dopamine receptors in the caudate nucleus play an important role in activating such pathway

Fig. 1. — Diagram of the neuronal connections within cerebellar cortex and deep cerebellar nuclei.

PC  =  Purkinje cell;
BC  =  Basket cell;
GOC = Golgi cell;
SC  =  Stellate cell;
GrC = Granular cell;
DCN = Deep cerebellar nuclei;
MF  =  Mossy fiber;
PF  =  Parallel fiber;
CF  =  Climbing fiber.

The cells in black are inhibitory and therefore presumably secrete GABA (modified from Eccles and Coll. [7]).
In fact, recent studies have shown that inhibition of dopamine (DA) receptors in the caudate nucleus by locally or systemically injected neuroleptics results in a dramatic fall in cGMP content in the rat cerebellar cortex [11, 12]. The effect of neuroleptics is antagonized by apomorphine, a direct stimulant of DA receptors, either systemically injected or directly injected into the caudate nucleus. Vice versa, the administration of apomorphine to normal rats induces an increase in cerebellar cGMP content [11, 12]. These results have been interpreted as an indication of a DA-activated stimulatory pathway between the caudate nucleus and the cerebellum.

Electrophysiological studies support the existence of a caudate-cerebellar pathway. In fact, stimulation of the caudate nucleus evokes two types of potentials in the contralateral cerebellar cortex: one type recorded in the lobulus simplex arriving via mossy fibers and another type in the paramedian lobule arriving via climbing fibers [13]. These pathways seem to have stations in different areas of the midbrain, such as substantia nigra, zona incerta, a region dorsal to the red nucleus, and eventually the pontine nuclei and superior olivary nucleus, where mossy and climbing fibers originate, respectively.

We have further investigated the importance of the dopamine receptors in the caudate nucleus in controlling the content of cGMP in the cerebellum (and presumably, Purkinje cell activity) and in mediating the changes induced by apomorphine on it. For this purpose we eliminated in rats striatal DA-sensitive adenylate cyclase with the intrastriatal administration of kainic acid, a rigid analog of glutamic acid, that is known to produce a degeneration of striatal perikarya but to leave intact afferent terminals or fibers on passage [14]. We now report that the disappearance of striatal DA-sensitive adenylate cyclase is paralleled by a marked decrease in cerebellar cGMP content and abolishes the effect of apomorphine on this nucleotide.

MATERIALS AND METHODS

Male Sprague Dawley rats (Charles River, Como, Italy) weighing 230–250 g were used.

Rats were anesthetized with Equithesin (3 ml/kg i. p.), and guide cannulae (stainless steel, 27 gauge) were stereotaxically implanted bilaterally in caudate nuclei (AP = 8.2, L = 2.8, DV = 1.5) [15]. Kainic acid or saline were administered through an injection needle (30 gauge stainless steel tubing) extending 1 mm beyond the cannula tip in a volume of 1 μl at a rate of 0.2 μl/30 sec, using a microburet syringe (Micrometric Instrument, Cleveland, Ohio). The rats were killed with a focused microwave beam [16].
and the cGMP and cAMP extracted from punches of cerebellar cortex with perchloric acid. The nucleotides were purified on alumina and Dowex columns [17] and assayed using the activation of cGMP or cAMP-dependent protein kinases [18].

Protein was measured according to Lowry and Coll. [19]: guanylate cyclase and adenylate cyclase activity were measured as previously described [68, 20]. Harmaline, apomorphine and isoniazid were injected peripherally by the route indicated in Fig. 2.

**Table 1**

Effect of the bilateral intrastriatal injection of kainic acid on striatal dopamine-sensitive adenylate cyclase activity and on the content of cGMP and cAMP in cerebellar cortex of rats

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>Striatal adenylate cyclase</th>
<th>Cerebellar cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basal activity</td>
<td>+ DA 5 × 10⁻⁴ M pmoi/mg protein/min</td>
</tr>
<tr>
<td>Solvent</td>
<td>119 ± 5.5</td>
<td>244 ± 14.2</td>
</tr>
<tr>
<td>Kainic acid (6 h)</td>
<td>109 ± 4.8</td>
<td>260 ± 16.1 (a)</td>
</tr>
<tr>
<td>Kainic acid (12 h)</td>
<td>93 ± 5.9 (a)</td>
<td>164 ± 12.5 (b)</td>
</tr>
<tr>
<td>Kainic acid (24 h)</td>
<td>78 ± 4.5 (a)</td>
<td>76 ± 15.5 (b)</td>
</tr>
<tr>
<td>Kainic acid (96 h)</td>
<td>10 ± 1.5 (a)</td>
<td>10 ± 2.1 (a)</td>
</tr>
</tbody>
</table>

Each point is the mean ± S. E. of five experiments.
(a) P < 0.05 when compared with the rats treated with solvent.
(b) P < 0.001 when compared with the rats treated with solvent.
RESULTS

Table 1 shows the time-course of the effect of bilateral administration of kainic acid (2 μg/striatum) on the DA-stimulated adenylate cyclase activity in the striatum and on cGMP and cAMP levels in the cerebellar cortex.

As expected from previous results [14], the intrastriatal injection of kainic acid resulted in a decrease of basal adenylate cyclase activity in striatal homogenates and in a complete loss of the activation of this enzyme by DA within 24 hours after treatment. At 4 days after kainic injection also basal activity of adenylate cyclase had disappeared.

The loss of DA-stimulated adenylate cyclase was associated with a parallel decrease in cerebellar cortex cGMP content, which was maximal (80 % loss) by 24 h and persisted up to 4 days after treatment. On the contrary, kainic acid caused no changes in cerebellar cAMP content.

We studied if the loss of striatal DA-sensitive adenylate cyclase interfered with the effect of apomorphine, harmaline and isoniazid on cerebellar cGMP content.

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Effect of apomorphine, harmaline and isoniazid on cerebellar cortex cGMP content of rats with both striata lesioned with kainic acid.

Kainic acid (2 μg/caudate), apomorphine (1 mg/kg s.c.), harmaline (6 mg/kg i.v.), and isoniazid (300 mg/kg i.p.) were injected 2 days, 15, 20 and 45 min before sacrifice, respectively.

Each point is the mean ± S. E. of 4 experiments. The asterisk indicates a P < 0.001 when compared to the saline-injected rats.
As shown in Fig. 2, apomorphine increased cerebellar cortex cGMP content in intact rats but failed to change this nucleotide in rats pretreated with kainic acid 3 days previously. On the contrary, harmaline, which increases cerebellar cyclic GMP content through the specific activation of the firing rate of climbing fibers [6] and isoniazid, which acts through a depletion of cerebellar GABA [21], increased the levels of cerebellar cortex cGMP in kainic acid-lesioned rats to the same extent as in control rats.

Finally the guanylate cyclase activity, assayed in homogenates of cerebellar cortex, was found to be equal in control rats as in rats lesioned with kainic acid.

**DISCUSSION**

The present results indicate that nigro-striatal DA system has the most important role in controlling cyclic GMP levels in the cerebellum. In fact, the loss of striatal adenylate cyclase after kainic acid is associated with an almost complete disappearance of the cerebellar cyclic GMP content and abolishes the effect of haloperidol and apomorphine on the levels of this nucleotide.

Since the loss of striatal adenylate cyclase by kainic acid reflects the destruction of post-synaptic DA-receptors [14], our results indicate that these receptors play a major role in regulating the cyclic GMP content in the cerebellum.

It is likely that kainic acid lesion eliminates a neuronal pathways from the striatum to the inferior olive and pontine nuclei, where climbing and mossy fibers originate, respectively. On the other hand, climbing fibers can still be activated directly by harmaline, while isoniazid, which acts through a depletion of cerebellar GABA, maintains its ability to increase cerebellar cyclic GMP content.

Kainic acid might destroy a neuronal chain in which the last neuron is either an excitatory one, projecting directly onto the climbing and mossy neurons, or an inhibitory one connected to the mossy and or climbing neurons through an inhibitory interneuron situated outside the striatum.

Considering that the effect of kainic lesion is equivalent to the blockade of DA receptors by haloperidol, and that this blockade results in a striatal cholinergic hyperactivity, one might suggest the model of Fig. 3 to represent the striocerebellar connections. According to this model DA would inhibit a cholinergic interneuron. The latter, in the absence of dopaminergic inhibition, would activate a GABA-ergic interneuron, which eventually inhibits a neuronal output from the striatum to the mossy and climbing fibers.

In any case, the neuronal-pathway from striatum to the mossy and climbing fibers seems to be the most important, if not the only neuronal
system activating cerebellar guanylate cyclase activity. This control system seems to be quite specific since kainic acid lesion is without effect on cerebellar cyclic AMP levels. Among the important problems to be solved is the identification of the neuronal pathway involved in the regulation of cerebellar activity. Conceivably, the microinjection of kainic acid, or of GABA mimetics and blockers, at different levels in the striato cerebellar pathway will help in clarifying this problem.

Results of such studies might be extended to clarify the control by the basal ganglia on the activity of other brain areas.

REFERENCES


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