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Interactions between central glutamatergic, catecholaminergic and cholinergic systems with regard to locomotor activity in monoamine-depleted mice

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Summary. Following injection of 5 μg of the competitive NMDA receptor antagonist AP-5 into the nucleus accumbens, but not following injection of the same dose into the dorsal striatum, a pronounced locomotor stimulation in monoamine-depleted mice was produced; the α-adrenoceptor agonist clonidine (1 mg/kg) administered ip caused a marked potentiation of an intraaccumbens AP-5 (2.5 μg) injection.

On the other hand, 10 μg of AP-5 combined with an ip injection of clonidine (1 mg/kg) caused a marked locomotor stimulation following local application into the dorsal striatum but not following application into the prefrontal cortex. Likewise, in combination with systemically administered clonidine, a substantial locomotor stimulation was observed after application of the muscarine receptor antagonist methscopolamine (62 μg) into the dorsal striatum but not into the prefrontal cortex.

This study suggests that NMDA receptors in the nucleus accumbens exert an inhibitory influence on locomotor activity. The dorsal striatum may also be involved in such control via NMDA and muscarinic receptors.

Keywords: Amino acids – Glutamate – Nucleus accumbens – Basal ganglia – Locomotion – Mouse

Introduction

It has been hypothesized that the striatal structures exert an inhibitory function on the thalamus and thus on the sensory input to the cerebral cortex as well as on arousal (Carlsson et al., 1988). The mesostriatal dopamine pathways are believed to be inhibitory on the striatum and thus to enhance the sensory input relayed by the thalamus to the cortex, whereas the corticostriatal glutamatergic pathway would have the opposite effect. Corticostriatal glutamatergic and

mesostriatal dopaminergic neurons appear to make synaptic contact with a common third neuron, ie with the dendritic spines pertaining to a GABAergic projection neuron (Freund et al., 1984). Thus, the corticostriatal glutamatergic and mesostriatal dopaminergic systems may operate independently of one another on the local level, sharing a common target neuron or final endpoint, influencing the thalamus in opposite directions. Hence, the behavioural consequences of a decreased activity in the corticostriatal glutamatergic neurons would be reminiscent of those produced by an increased activity in the mesencephalostriatal dopaminergic neurons, ie increased wakefulness and locomotor stimulation. In support of this hypothesis we discovered that both competitive and non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists are capable of inducing locomotion in mice even after the virtually complete depletion of catecholamine stores, brought about by reserpine and α-methyltyrosine pretreatment. Moreover, we observed that partial inhibition of glutamatergic activity, induced by subthreshold doses of NMDA receptor antagonists, interacted in a synergistic manner with subthreshold doses of dopaminergic agonists, and disclosed a strong stimulatory potential of α_2-adrenergic receptor agonists as well as of muscarinic receptor antagonists (for refs. see Carlsson et al., 1991).

To analyze the role of the basal ganglia in this context, further efforts are focussed on local application of various receptor ligands. This paper describes some initial experiments aimed at comparing the dorsal striatum, the nucleus accumbens and the prefrontal cortex of mice with regard to locomotor effects following local injections of an NMDA antagonist or a muscarinic antagonist. In some experiments the addition of a systemically administered α_2-adrenergic receptor agonist was evaluated.

Material and methods

Animals

Male albino mice of the NMRI strain weighing 20–25 g were purchased from ALAB, Sollentuna.

Drugs

Reserpine (Ciba-Geigy AG) was dissolved in a few drops of glacial acetic acid and a 5.5% glucose solution. Ketamine hydrochloride (Sigma), α-methyl-para-tyrosine methylester hydrochloride (α-MT; Sigma), clonidine hydrochloride (Boehringer Ingelheim) and methscopolamine nitrate (Pharmacia) were dissolved in physiological saline. The commercially obtained solution of xylazine chloride (Rompun vet.; Bayer) was diluted to 0.75 mg/ml with physiological saline. Ketamine, xylazine, α-MT and clonidine were injected ip in a volume of 10 ml/kg. Reserpine was given ip in a volume of 20 ml/kg.

DL-2-amino-5-phosphonopentanoic acid (free acid; AP-5; Sigma) was dissolved in 1 M NaOH and diluted with distilled water. 20 μg methylene blue (Sigma) was added to each μl of the AP-5 solution to make it possible to locate the injection sites anatomically. The solution was finally adjusted to neutral pH and isotonicity by adding NaOH and NaCl, respectively.

Surgery

Stereotaxic surgery was performed under ketamine (150 mg/kg) and xylazine (7.5 mg/kg) anaesthesia. Guide cannulas (∅ 0.60 mm, length 17 mm) were implanted unilaterally and

fixed to the skull, the tips of the cannulas reaching just above the surface of the brain. The animals were allowed at least three days' recovery following surgery.

Using injection cannulas (⌀ 0.40 mm) the competitive NMDA receptor antagonist AP-5 or the muscarine receptor antagonist methscopolamine was injected in a volume of 0.1 (Figs. 2c, 3ab), 0.2 (Figs. 2ab) or 0.5 μl (Fig. 1) into the dorsal striatum, nucleus accumbens or prefrontal cortex (see below) of the freely moving animals.

After completion of the locomotor registrations the brains were sectioned on a freezing sledge microtome and only animals with stained injection sites within the following coordinates, according to the atlas of Slotnick and Leonard (1975), were included in the study: The dorsal striatum (in the nucleus caudatus-putamen bordering on the globus pallidus): A/P 0.0–0.4 mm posterior to bregma, L 2.0–2.6 (on the right side) and V 2.6–3.4 mm. The nucleus accumbens: A/P 0.4–1.1 mm anterior to bregma, L 0.8–2.0 (right) and V 3.8–4.6 mm. The prefrontal cortex: A/P 1.8–2.2 mm anterior to bregma, L 0.8–2.4 (right) and V 1.4–2.4 mm.

Locomotor registration

Locomotor activity of monoamine-depleted animals treated with AP-5 was measured in circular tracks as previously described (Carlsson et al., 1991). In experiments with intact animals, or monoamine-depleted animals treated with methscopolamine, locomotor activity was measured in rectangular plexiglass cages by means of electronic motility meters. One hour following reserpine administration and throughout the experiment the ambient temperature was held at 26°C. In addition, monoamine-depleted animals were kept warm on electric pads until the locomotor registration commenced.

Statistics

Mann-Whitney U-test was used throughout for comparisons between groups.

Results

A unilateral injection of the competitive NMDA receptor antagonist AP-5 (10 μg) into the dorsal striatum of *intact* mice significantly increased locomotor activity (Fig. 1).

A pronounced stimulation of locomotor activity was also induced when 5 μg of AP-5 was injected into the nucleus accumbens of *monoamine-depleted* mice (Fig. 2a); no stimulatory effects were registered following administration of

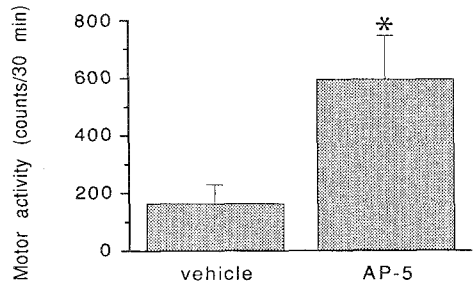

Fig. 1. Effects of AP-5 injected into the dorsal striatum of intact mice. The animals were habituated to the motility meters during 30 min. preceding the administration AP-5. After the injection of AP-5 (10 μg) or vehicle the animals were immediately placed in the motility meters and locomotor activity was registered during 30 min. Shown are the means ± s.e.m., $n = 3$. *$p = 0.049$ vs vehicle

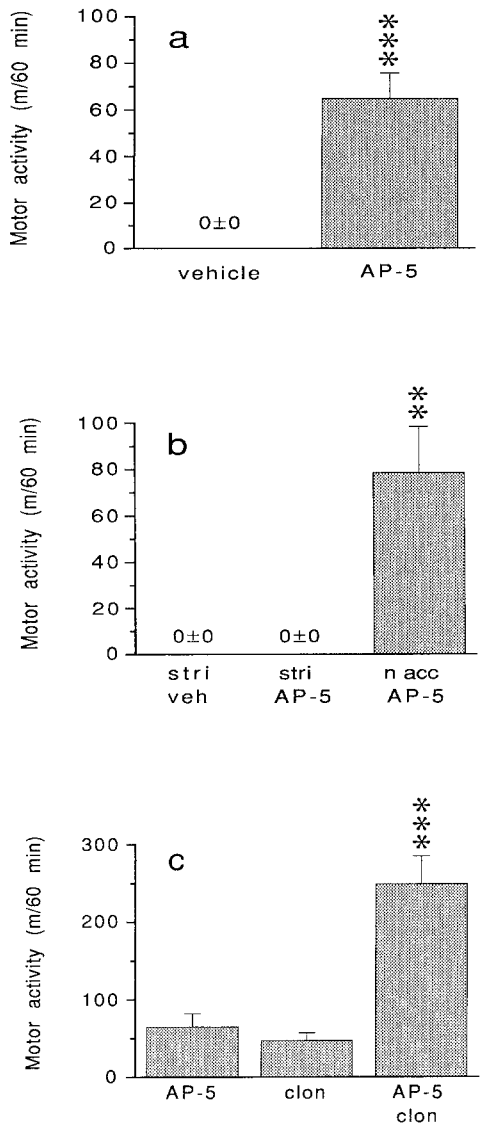

Fig. 2. In the experiments shown in Figs. 2 and 3 the mice were monoamine-depleted by means of pretreatment with reserpine (10 mg/kg) 18 hours and α-MT (500 mg/kg) 2 hours before the registration of locomotor activity commenced. Note that the scales differ in the various figures. **a** Effects of AP-5 injected into the nucleus accumbens. Ten min. after the AP-5 (5 μg) or vehicle injection, the animals were placed in the circular tracks and locomotor activity was registered during 60 min. Shown are the means ± s.e.m., $n = 6$–8. ***$p = 0.0006$ vs vehicle. **b** Effects of AP-5 injected into the nucleus accumbens or dorsal striatum. Ten min. after the AP-5 (5 μg) or vehicle injection, the animals were placed in the circular tracks and locomotor activity was registered during 60 min. Shown are the means ± s.e.m., $n = 4$–6. **$p = 0.0039$ vs AP-5 injected into the dorsal striatum. **c** Effects of a low dose of AP-5 injected into the nucleus accumbens, given alone or in combination with ip administered clonidine. Ten min. following the injection of AP-5 (2.5 μg) and immediately following the administration of clonidine (1 mg/kg) the animals were placed in the circular tracks and locomotor activity was measured during 60 min. Shown are the means ± s.e.m., $n = 9$–11. ***$p <$ or $= 0.001$ vs AP-5 or clonidine alone.

this dose of AP-5 into the dorsal striatum (Fig. 2b). An ip injection of the α-adrenoceptor agonist clonidine (1 mg/kg) caused a marked potentiation of 2.5 μg of AP-5 applied into the nucleus accumbens (Fig. 2c).

Furthermore, in combination with clonidine (1 mg/kg ip) 10 μg of AP-5 caused a marked locomotor stimulation in monoamine-depleted mice if applied into the dorsal striatum, but not if applied into the prefrontal cortex (Fig. 3a).

By analogy, the dorsal striatum was compared to the prefrontal cortex with respect to the effects of the combined treatment with systemic clonidine and intracerebral administration of the muscarine receptor antagonist methscopolamine (62 μg). Also in this case the intrastriatal but not the intracortical injection resulted in a substantial stimulation of locomotor activity (Fig. 3b).

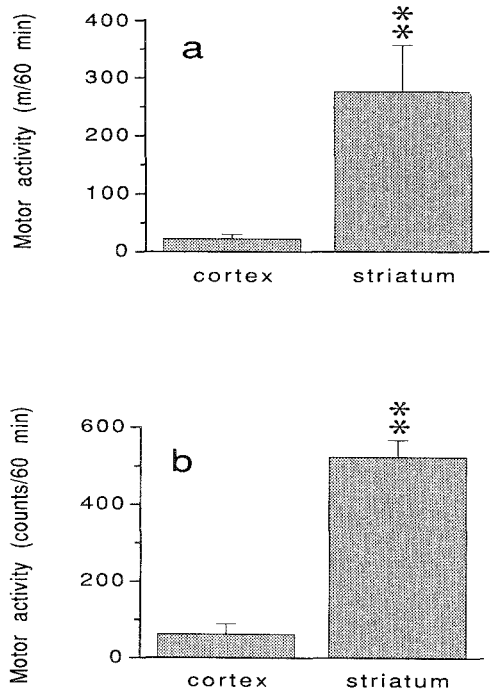

Fig. 3. a Effects of AP-5 injected into the dorsal striatum or prefrontal cortex in combination with ip administered clonidine. Ten min. following the injection of AP-5 (10 μg) and immediately following the administration of clonidine (1 mg/kg) the animals were placed in the circular tracks and locomotor activity was measured during 60 min. Shown are the means ± s.e.m., $n = 6–8$. $^{**}p = 0.002$ vs the prefrontal cortex. **b** Effects of methscopolamine injected into the dorsal striatum or prefrontal cortex in combination with ip administered clonidine. Immediately following the administration of methscopolamine (62 μg) and clonidine (1 mg/kg) the animals were placed in the motility meters and locomotor activity was measured during 60 min. Shown are the means + s.e.m., $n = 5–6$. $^{**}p = 0.0062$ vs the prefrontal cortex

Discussion

These results suggest that the nucleus accumbens is important in the regulation of locomotor activity via NMDA receptors, which appear to be inhibitory in

this respect. These results are not surprising, given the wealth of evidence indicating a role for this nucleus in the control of locomotor activity by dopamine (Johnels, 1982; Pijnenburg et al., 1973). However, the dorsal striatum may also be involved in such control, as suggested by the experiments where AP-5 or methscopolamine was administered locally in the presence of systemically administered clonidine. In further support of this notion, Schmidt (1986) has observed behavioural stimulation following injection of AP-5 into rat anterodorsal striatum. The impact of this structure for locomotor activity appears to be less powerful, though, than that of nucleus accumbens, as indicated by the results with local application of 5 μg of AP-5 in the absence of clonidine. No evidence for a prominent role of the prefrontal cortex in the action of AP-5 or methscopolamine on locomotor activity was obtained in the present study.

The studies with regional application reported here need to be extended to other receptor ligands, including non-NMDA receptor antagonists, other brain regions and other experimental conditions with different baseline activity levels in order to give a more complete picture. However, already at this stage it is apparent that nucleus accumbens glutamate receptors play an important role in regulating psychomotor functions.

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