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Akinesia due to catecholamine depletion in mice is prevented by caffeine. Further evidence for an involvement of adenosinergic system in the control of motility

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Abstract—The administration of reserpine with α-methyl-*p*-tyrosine (2·5 and 200 mg kg^{-1} i.p., 24 and 3 h before the test, respectively) induced a marked akinesia in mice. This effect was significantly and dose-dependently reversed by the methylxanthine, caffeine. The anti-akinetic effect of caffeine within a pattern of catecholamine depletion has been interpreted as a dopamine mimetic activity of this drug. The possible involvement of the adenosine system in this effect of caffeine is discussed.

Several interactions seem to exist between the adenosine and dopamine systems in the central nervous system (CNS). Local 5-*N*-ethylcarboxamide adenosine (NECA, a stable adenosine analogue), inhibits apomorphine-induced effects in the nucleus caudatus of rats (Green et al 1982). In rabbits, the blockade of adenosine receptors induces a dopamine-like stereotyped behaviour which is prevented by NECA but not by haloperidol (Caporali et al 1987; Popoli et al 1989). In mice, several similarities between the behavioural effects of adenosine agonists and dopamine antagonists have been shown (Haeffner et al 1989). Adenosine analogues inhibit spontaneous motility in several animal species, and this effect seems to occur at the CNS level (Spealman & Coffin 1986; Durcan & Morgan 1989).

On the whole, these data indicate that the adenosine system may play a role in the control of movements, probably through a tonic inhibition of the dopaminergic neurotransmission. On the other hand, if adenosine receptor agonists prevent dopamine-like stereotypy, it is reasonable to suppose that the antagonists at these receptors (e.g. the methylxanthine caffeine), should antagonize the effects of a reduced dopaminergic neurotransmission.

To further assess the possible relevance of the adenosine system in the control of movements, we investigated the influence of caffeine in the akinesia induced by α-methyl-*p*-tyrosine (α-MPT, a tyrosine hydroxylase inhibitor) plus reserpine (a catecholamine depletor) in mice.

Materials and methods

Animals. Adult male Swiss mice, 25–30 g, were kept under standardized humidity, temperature and lighting conditions (on a constant 0700–1900 h light-dark schedule), with free access to water and food.

Experimental procedure. Eight groups of ten mice each were treated i.p. as follows: 0·9% NaCl (saline) (1 mL kg^{-1}, 30 min before the test), caffeine (10, 25 and 50 mg kg^{-1}, 30 min before the test), reserpine + α-MPT (2·5 and 200 mg kg^{-1}, 24 and 3 h before the test, respectively), reserpine + α-MPT + caffeine (10, 25 and 50 mg kg^{-1}).

Each group was observed over 30 min. Every 5 min, immobility periods were recorded for each animal. Immobility was defined as absence of either locomotor or non-locomotor (e.g. sniffing, grooming, head movements) activity. For each group, the mean time spent in immobility every 5 min was calculated.

The data were statistically analysed using the 1-way ANOVA Dunnett's test.

Results and discussion

As shown in Table 1, the lower dose of caffeine induced a non-significant increase in spontaneous motility with respect to saline. This effect decreased with the intermediate dose of caffeine and disappeared completely at 50 mg kg^{-1}.

The animals treated with reserpine + α-MPT showed a very marked akinesia (mean immobility period of 4·32/5 min). This effect was significantly reversed by caffeine (see Table 1). As also observed in control animals, the lowest caffeine dose was the most effective in counteracting reserpine + α-MPT-induced akinesia.

These data demonstrate that caffeine markedly and dose-dependently prevents the appearance of akinesia within a pattern of catecholamine depletion. Since this catecholamine depletion is not selective for dopamine, the possibility exists that the anti-akinetic effect of caffeine may be due to an action on catecholamine neurotransmitter systems other than the dopamine system. However, it is well known that dopamine is essential for motor activation. For this reason, the lack of endogenous dopamine is likely to be a key phenomenon in the genesis of akinesia. Thus, the anti-akinetic activity of caffeine within this pattern may be interpreted as a dopaminomimetic effect. Since caffeine does not displace the binding of [^{3}H]spiperone to striatal membranes, the dopamino-mimetic activity of this drug seems not to be linked to a direct stimulation of dopamine receptors (Watanabe & Uramoto 1986). Thus, caffeine may be reasonably supposed to facilitate dopaminergic neurotransmission indirectly. In agreement with a number of studies indicating close connections between the adenosine and dopamine systems, we suggest that a removal of the tonic inhibition exerted by adenosine may underlie the dopaminomimetic activity of caffeine; caffeine may unbalance the dopa-

Table 1. Influence on mice of caffeine on reserpine + α-methyl-*p*-tyrosine (α-MPT)-induced akinesia (n = 10).

Treatment (mg kg^{-1} i.p.)	Time (min/5 min) animals were immobile (mean ± s.e.m.)
Saline	1·2 ± 0·6
Caffeine (10)	0·52 ± 0·3
Caffeine (25)	0·82 ± 0·4
Caffeine (50)	1·1 ± 0·7
Reserpine (2.5) + α-MPT (200)	4·32 ± 0·5°
Reserpine + α-MPT + caffeine (10)	0·1 ± 0·1†*
Reserpine + α-MPT + caffeine (25)	0·5 ± 0·2†
Reserpine + α-MPT + caffeine (50)	0·86 ± 0·3†

°$P < 0·001$ vs saline, †$P < 0·0001$ vs reserpine + α-MPT, *$P <= 0·05$ vs reserpine + α-MPT + caffeine 50 mg kg^{-1}, according to ANOVA Dunnett's test.

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mine-adenosine relationship, promoting a more marked manifestation of the dopaminergic effects.

In agreement with recent suggestions pointing to a specific purinergic contribution to the neurological function in the basal ganglia (Jarvis et al 1989), the hypothetical role of adenosine in the modulation of movements seems to be confirmed.

Further studies are needed to investigate whether the drugs affecting the adenosine system may be useful in the treatment of dopaminergic disorders.

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Response of canine cerebral arteries to endothelin-1

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Abstract—The effects of endothelin-1 (10^{-10}–10^{-7} M) were isometrically recorded in 4 mm cylindrical segments from the middle cerebral artery of dogs. Cumulative application of endothelin-1 produced marked, sustained contraction of arteries in a concentration-dependent-manner, the maximal response being about 2·6 times higher than that achieved with KCl (50 mM). The contraction by endothelin-1 was unaffected either by endothelium removal or by the cyclo-oxygenase inhibitors indomethacin (10^{-6} M) and meclofenamate (10^{-6} M). In a Ca^{2+}-low (25 μM) solution the endothelin-1-induced arterial contraction was decreased. Therefore, the cerebral vasoconstriction induced by endothelin-1 could be caused by activation of specific receptors located on smooth muscle cells which would lead to the influx of extracellular calcium and vascular musculature contraction.

It has been demonstrated that the endothelium plays a main role in the regulation of vascular tone by releasing relaxing and contracting factors (Furchgott & Vanhoutte 1989). Endothelin is an endothelium-derived 21 amino acid peptide which has been isolated and sequenced by Yanagisawa et al (1988b). This peptide is similar among some mammalian species: human and porcine endothelin have the same amino acid sequence (Itoh et al 1988), whereas rat endothelin has 76% homology, and a slightly different pharmacological potency (Yanagisawa et al 1988a). Endothelin-1 produces a potent, long-lasting constrictor effect in several vascular beds in-vivo and in-vitro (Hughes et al 1988; Yanagisawa et al 1988b; Eglen et al 1989; Hinojosa-Laborde et al 1989; Kasuya et al 1989; King et al 1989). However, endothelin-1 appears to be a vasoconstrictor selective for some vascular beds (Clozel & Clozel 1989) and its effects are not limited to vasoconstriction as it can also produce vasodilation in-vivo and in-vitro (de Nucci et al 1988; Folta et al 1989; Lippton et al 1988).

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Few experiments have been reported on the effects of endothelin-1 on cerebral blood vessels and its possible involvement in the regulation of the cerebral circulation has not been determined.

The present experiments were designed to examine the effects of endothelin-1 on canine isolated cerebral arteries and the role of the endothelium, prostanoids and extracellular calcium on these effects.

Materials and methods

Eight mongrel dogs, 18–27 kg, were anaesthetized with sodium pentobarbitone injected intravenously, and exsanguinated. The brain was then carefully removed and both middle cerebral arteries were dissected out and cut into cylindrical segments 4 mm in length and approximately 950 μm in external diameter. Two stainless-steel pins, 150 μm in diameter, were introduced through the arterial lumen. One pin was fixed to the organ bath wall while the other was connected to a strain gauge. The recording system included a Universal transducing cell (UC3), a Statham microscale accessory (U15) and a Beckman type RS recorder. Each arterial segment was set up in a 6 mL bath containing modified Krebs-Henseleit solution with the following composition (mM): NaCl 115; KCl 4·6; KH_2PO_4 1·2; $CaCl_2$ 2·5; $NaHCO_3$ 25; $MgSO_4$. 7 H_2O 1·2; glucose 11·1 and Na_2H_2 EDTA 0·01. The solution was equilibrated with 95% O_2- 5% CO_2 to give a pH of 7·3 to 7·4 and temperature was held at 37°C. Arterial segments were equilibrated at a passive tension of 1·5 g for 1 h.

Response of vascular segments to endothelin-1 (10^{-10}–10^{-7} M) was determined in a cumulative manner. This was carried out in intact, non-treated arteries (control), in arteries without endoth-