Pharmacology Biochemistry & Behavior, Vol. 19, pp. 535–541, 1983. © Ankho International Inc. Printed in the U.S.A.

On the Mechanism by Which Methylxanthines Enhance Apomorphine-Induced Rotation Behaviour in the Rat

BERTIL B. FREDHOLM, MARIO HERRERA-MARSCHITZ, BROR JONZON, KARIN LINDSTRÖM AND URBAN UNGERSTEDT

Department of Pharmacology, Karolinska Institutet, Box 60 400, S-104 01, Stockholm, Sweden

Received 24 February 1983

FREDHOLM, B. B., M. HERRERA-MARSCHITZ, B. JONZON, K. LINDSTRÖM AND U. UNGERSTEDT. *On the mechanism by which methylxanthines enhance apomorphine-induced rotation behaviour in the rat.* PHARMACOL BIOCHEM BEHAV **19**(3) 535–541, 1983.—Methylxanthines, such as caffeine and theophylline, potentiate the rotation behaviour induced by dopamine receptor agonists in rats with unilateral lesions of the nigro-striatal pathway. In the present study we have examined the possibility that interaction with central adenosine mechanisms could influence rotation behaviour. Under *in vitro* conditions adenosine and N^6-phenylisopropyl-adenosine (PIA) stimulate cyclic AMP accumulation. This effect was enhanced by the phosphodiesterase inhibitor rolipram, but blocked by alkylxanthines such as caffeine, theophylline and, particularly, 8-phenyl-theophylline. Rotation behaviour induced by apomorphine (0.05 mg/kg), was inhibited by PIA and rolipram and by a low dose of the adenosine deaminase inhibitor EHNA (2 mg/kg). By contrast, theophylline and 8-phenyl-theophylline caused a potentiation. The former drug stimulated rotation behaviour per se, while the latter did not. 8-Phenyl-theophylline entered the brain poorly and its concentration in brain it was less than 1/10 of theophylline. It is concluded that theophylline does not potentiate rotation behaviour secondarily to inhibition of phosphodiesterase. Antagonism of endogenous adenosine may partly explain the effect of methylxanthines. Possibly, some as yet unknown mechanism may also contribute to the effects of xanthine-derivatives on rotation behaviour.

Cyclic AMP Adenosine Dopamine receptors Alkylxanthines/pharmacokinetics

METHYLXANTHINES such as caffeine and theophylline are known to increase spontaneous motility in rats [3, 21, 33]. In rats with unilateral lesions of the nigro-neostriatal dopamine pathway apomorphine and L-DOPA have been shown to induce contralateral rotation [35]. It was shown by Fuxe and Ungerstedt [19] that caffeine and theophylline induce longlasting rotation behaviour of a similar kind as that produced by the dopamine receptor stimulating agents. Moreover, the methylxanthines were found to enhance the rotation induced by apomorphine, L-DOPA and pirebidil (Et-495). The rotation induced by amphetamine, which occurs in the opposite direction, was also potentiated by caffeine [19]. Dopamine is capable of stimulating cyclic AMP formation in the rat striatum [22], and the methylxanthines are phosphodiesterase inhibitors [6]. The potentiation of rotation behaviour was therefore assumed to be secondary to a potentiated cyclic AMP accumulation in relevant effector cells [19]. This finding was supported when it was shown that a more potent phosphodiesterase inhibitor, isobutylmethylxanthine, was more potent as a stimulator of rotation behaviour [11] and by the finding that intrastriatal injections of cyclic nucleotides cause rotation [27]. Other evidence was more difficult to reconcile with the hypothesis. Thus, already Fuxe and Ungerstedt [19] showed that the potent phosphodiesterase inhibitor papaverine did not potentiate rotation behaviour, and it was later shown that it may actually inhibit such behaviour [11]. Furthermore, still other potent phosphodiesterase inhibitors like ICI 63197 actually inhibited the rotation [1]. It was also found that various ergot-derivates, which show weak and inconsistent stimulation of cyclic AMP formation, caused rotation behaviour [17,18]. This rotation was markedly potentiated by isobutylmethylxanthine. Finally, it was found that methylxanthines in the doses needed to produce rotation behaviour did not reach concentrations in the brain sufficient to cause significant inhibition of phosphodiesterase activity [11].

The methylxanthines are not only inhibitors of cyclic AMP hydrolysis but they also antagonize the effects of adenosine on specific adenosine receptors [10]. Such an inhibition is found at considerably lower concentrations than those required to block phosphodiesterase. It has therefore been suggested that at least some of the actions of methylxanthines may be caused by adenosine antagonism [2, 8, 10]. For example, recent data obtained in the rat hippocampus have provided evidence that the action of methylxanthines on neuronal excitability is in fact due to this latter mechanism of action [7].

We have therefore further studied the mechanism behind

the effect of methylxanthines on apomorphine induced rotation behaviour in the rats. To this end we have used a number of relatively selective pharmacological tools. Rolipram, ZK 62711, is a potent inhibitor of cyclic AMP hydrolysis lacking effects on adenosine levels or adenosine effects [29]. 8-Phenyl-theophylline is a theophylline derivative with an increased potency as an antagonist of adenosine receptors but with a reduced potency as a phosphodiesterase inhibitor [7,31]. Erythro-9-(2-hydroxy-3-nonyl)adenine, EHNA, is an antagonist of adenosine deaminase [28] which is able to enhance the levels of endogenous adenosine in some tissues and body fluids [14]. Phenylisopropyladenosine, finally, is an adenosine analogue with a high potency on certain types of adenosine receptors [26, 30, 32, 38]. Using these drugs and more classical methylxanthines we find that the ability to influence the rotation behaviour of rats induced by apomorphine may be related to the ability to antagonize the effects of endogenous adenosine, but not to the ability to block cyclic AMP [12,16].

METHOD

Materials

The following compounds were used: Theophylline, as the ethylene-diamine salt, and caffeine from ACO (Sweden); 3-isobutyl-1-methylxanthine from Calbiochem; Rolipram, ZK 62711, a gift from Schering AG, Berlin; EHNA a gift from Burroughs-Wellcome, Research Triangle Park, NC; L-N^6-phenyl-isopropyl-adenosine (L-PIA) from Boehringer, Mannheim; 8-phenyl-theophylline from Calbiochem (dissolved in an aqueous solution of tetra-phenyl-boronate). All drugs were injected IP in a volume of less than 1 ml, using appropriate solvents as controls.

[^{14}C]-Adenosine (45 mCi/mmol) and 2,8 [^{3}H]-adenosine (36.2 Ci/mmol) were obtained from New England Nuclear. [^{3}H]-Adenosine 5-triphosphate (19 Ci/mmol) and [^{14}C]-adenosine 5-monophosphate (51 mCi/mol) were obtained from the Radiochemical Centre, Amsterdam. Other chemicals were reagent grade from various suppliers.

In Vitro *Studies*

Male Sprague Dawley rats (180–250 g) were killed by decapitation, the hypothalamus rapidly dissected out and sliced (0.26 mm thickness) in a McIlwain tissue chopper. The slices were preincubated for 15+45 min in fresh Krebs Ringer Bicarbonate buffer, with the following composition (mM): NaCl 118, KCl 4.85, KH_2PO_4 1.15, $MgSO_4$ 1.15, $CaCl_2$ 2.5, $NaHCO_3$ 14.7 pH 7.5 containing 11.1 mM glucose, and gassed with 95% O_2 and 5% CO_2. In the experiments with decreased pH the buffer had the following composition (mM): NaCl 130.4, KCl 4.85, KH_2PO_4 1.15, $MgSO_4$ 1.15, $CaCl_2$ 2.5, $NaHCO_3$ 2.3, Glucose 11.1. Three slices were placed in a 2 ml plastic tube containing 1 ml fresh buffer. Drugs were added in a total volume of 50 μl. After incubation for 10 min the incubation was stopped by addition of 100 μl 100% trichloracetic acid and by placing the tubes in an ice-water bath. The slices were homogenized by sonication. The protein free supernatants after centrifugation were extracted three times with water saturated ether and frozen until assay for cyclic AMP content by the method of Brown *et al.* [4]. The protein content of pellets was determined by Biuret reagent using bovine serum albumin as standard.

Release of purines was studied in 20 similar slices, preincubated with 20 μCi (^{14}C)adenosine, for 40 min at 37°C. The slices were placed in superfusion-chambers [15]. After washing for 45 min, 5 min samples were taken. The distribution of radioactivity was studied by TLC on prefabricated sheets of PEI-cellulose (Merck, Darmstadt) using n-butanol:actic acid:water (2:1:1) as running phase. Those parts of the chromatogram that corresponded to the position of unlabelled carriers were cut out and the radioactivity determined by liquid scintillation spectrometry.

Uptake and metabolism of purines were studied by superfusing hypothalamic slices with pulses of [^{3}H]-ATP, [^{14}C]-AMP and [^{3}H]-adenosine. The superfusates were chromatographed on TLC as described above.

Brain and Plasma Levels of Methylxanthines

Male Sprague Dawley rats weighing 150–250 g were used. In most of the experiments the rats received an IP injection of the respective methylxanthine in 0.5–1.0 ml. In some experiments the drugs were given orally by gavage. At indicated times the rats were killed by decapitation. Blood was collected from the severed neck and rapidly centrifuged. The plasma was deproteinized with 1:10 volume 4 M perchloric acid. When 8-phenyl-theophylline was to be determined, 70 percent acetonitrile was added. The brains were removed and the hippocampus, hypothalamus, cerebral cortex, striatum and cerebellum dissected out. These brain regions were weighed and homogenized in 20 volumes 0.4 M perchloric acid. An aliquot of the supernatant after centrifugation was neutralized with 1:10 volume 4 M KOH and 1:20 volume 1 M Tris. The samples were stored at −20°C until assay. Before assay the samples were centrifuged for 5 min in a Beckman Microfuge at approximately 10,000×g. The contents of the methylxanthines were assayed by reversed phase HPLC, using a Waters Model 440 solvent delivery system, a UK6 injector, a Model 400 UV-spectrophotometer and a 30 cm μ-Bondapack C_{18} columns with a 5 cm guard column. The solvent system used for the assay of theophylline and caffeine was 0.02 M phosphate buffer, pH 3.5, acetonitrile (90:10); for isobutylmethylxanthine-phosphate buffer, acetonitrile (80:20); for 8-phenyl-theophylline phosphate buffer, acetonitrile, methanol (60:20:20). Under these conditions their respective retention times (min) were: theophylline 5.6, caffeine 9.4, isobutyl-methylxanthine 7.7, 8-phenyl-theophylline 9.2 The recovery (percent) of the methylxanthines from brain (10 nmol added during homogenization) were theophylline 71, IBMX 80, caffeine 81 and 8-phenyl-theophylline 64. Extraction into toluene or propropanol/chloroform gave considerably lower recoveries. Addition of acetonitrile (10 percent) to the PCA slightly enhanced recovery. Recovery (percent) from plasma was: Theophylline 82, caffeine 81, isobutyl-methylxanthine 86 and 8-phenyl-theophylline 56.

Studies of Rotation Behaviour

Sprague Dawley male rats weighing between 160 and 180 g were housed four to a cage with access to rat chow and water ad lib. They were maintained in a controlled environment on a 12 hr light-dark cycle when they were not used in experiments.

The rats were placed in a stereotaxic David Kopf frame and anaesthetized by letting them breathe freely a mixture of air and halothane. The rat skull was oriented according to the König and Klippel atlas [23]. Eight μg of 6-OHDA (calculated as the base) was injected in 4 μl of saline solution

during 4 min into the left bundle of the dopamine axons just anterior to the mesencephalic dopamine cell bodies into the area ventralis tegmenti. The coordinates were 4.4 mm behind and 1.2 mm lateral to Bregma and 7.8 mm below the brain surface measured from the pia matter. This lesion extensively denervates the forebrain dopamine innervated areas according to our previous studies [34]. The injections were made with a blunt steel needle with a diameter of 0.4 mm.

In order to select the successfully denervated animals, rats were challenged with a very low dose of apomorphine (0.05 mg/kg SC) 2 weeks after the 6-OHDA injection, and then this testing was repeated with at least one week's interval until rats showed a clear and stable pattern of rotation. Only rats showing a two-peaks pattern of rotation (i.e., an initial rapid increase in rate of turning, followed by a period of a lower rate of turning control and, finally, a second peak of high rate of rotation—see Figs. 4 and 5) were used in the experiments, since we have previously found that rats with this pattern have the lowest concentrations of dopamine in the striatum on the 6-OHDA injected side [36,37]. Rotation was recorded in a rotometer. The rats were connected to the movement detector by a steel wire attached to a harness consisting of a 15 mm wide cloth band fitted over the chest of the animals. They were allowed a half an hour habituation period in the rotometers before every drug experiment. Each 180° right or left turn gave a count that was fed into a PCS super pack 180 microcomputer for intermediate storage and display. After completion of the experiments the data were transferred via a RS-232 interface to a Wang 2200 minicomputer for permanent storage on diskettes. The rotational behaviour of each individual was plotted as number of 360° turns/min for the entire duration of the induced behaviour.

An A-B-A block-design (where A refers to a control period and B to a period of drug treatment, see Figs. 4 and 5) was used to study the ability of successive increasing dose of theophylline (10 and 25 mg/kg), Rolipram (1 and 5 mg/kg), 8-phenyl-theophylline (5, 10 and 20 mg/kg), L-phenylisopropyl-adenosine (0.2, 2.0 and 5.0 μmol/kg) and EHNA (2,5 mg/kg) to modify the rotational behaviour induced by the standard dose of apomorphine of 0.05 mg/kg SC. An interval of at least one week was used between the control and interaction trials. During the interaction trials, drugs were injected IP 30 min before apomorphine.

Apomorphine HCl (Apoteksbolaget, Sweden) was dissolved in warm physiological saline and given in a dose referred to the free base and injected subcutaneously in a volume of 1 ml/kg into the left flank. Theophylline (Teofylamin, ACO) was diluted from injection ampoulles, Rolipram, 8-phenyl-theophylline and EHNA were diluted to reach the concentration of 5 ml/kg body weight and injection IP.

Mean and S.E.M. were calculated and expressed in terms of % of the control values. F-ANOVA test was used to analyse the dose-response interactions, followed by paired Student's *t*-test for individual comparisons.

RESULTS

In Vitro *Experiments*

In order to characterize the drugs used in the *in vivo* experiments we determined their effects on the accumulation of cyclic AMP in rat hypothalamic slices. Hypothalamic slices were used in these experiments because they gave more reproducible measurements of endogenous cyclic AMP than did striatal or hippocampal slices. However, principally similar findings were obtained also in slices from these brain

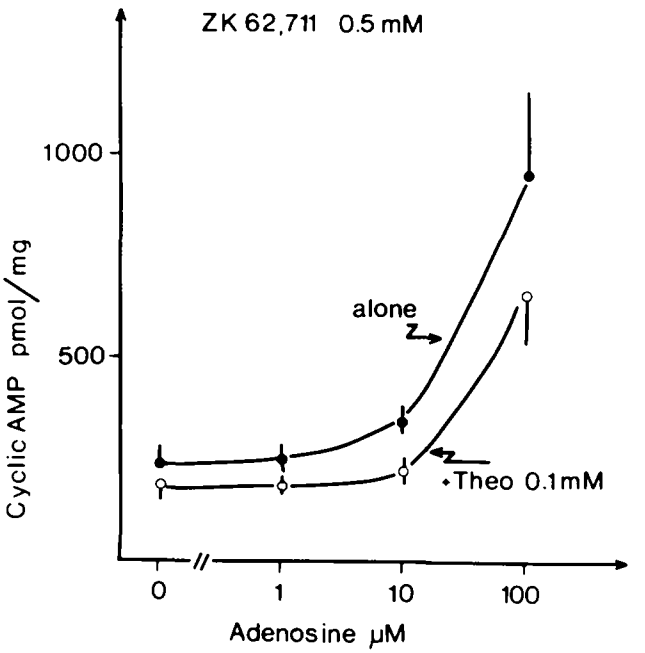

FIG. 1. Dose-dependent increase in cyclic AMP content of hypothalamic slices of the rat in the presence and absence of 0.1 mM theophylline. Mean±s.e.m. n=8.

regions (results not shown). The basal level of cyclic AMP was 19±2 pmol/mg protein (mean±s.e.m., n=20). Theophylline and caffeine produced a concentration dependent decrease in cyclic AMP content. At 1 mM concentration the levels were 7±3 and 13±4 pmol/mg, respectively. Adenosine, 2-chloro-adenosine and N^6-phenyl-isopropyl-adenosine dose-dependently increased cyclic AMP levels. At 0.1 mM concentration of these drugs the corresponding levels of cyclic AMP were 123±15, 166±23 and 184±12 pmol/mg, respectively. The effect of adenosine (0.1 mM) was dose-dependently inhibited by theophylline and caffeine. Half-maximal inhibition was seen at about 3×10^{-5} M for both drugs. 8-Phenyl-theophylline was some 30 times more potent. By contrast, the non-methylxanthine phosphodiesterase inhibitor, rolipram, ZK 62,711, at 5×10^{-5} M concentration caused a significant enhancement of the effect of adenosine and an elevation of the basal cyclic AMP level (35 vs. 4-fold). The effect of ZK 62,711 was still more pronounced at 0.5 mM concentration when the basal level of cyclic AMP was increased more than 10-fold. Even under these circumstances adenosine caused a dose-dependent increase in cyclic AMP levels, which was antagonized by theophylline (Fig. 1). The effect of adenosine (10^{-4} M) was significantly lower at an acid pH (6.6) compared to normal pH (7.4) (642±202 and 1257±270 pmol/mg, respectively, in the presence of 5×10^{-3} M ZK 62,711). The effect of theophylline was inhibited at low pH.

In three separate experiments hypothalamic slices were labelled with [^{3}H]-adenine and thereafter superfused. When EHNA was included in the medium at a concentration of 1 μM there was a slight increase in the amount of radioactivity recovered as adenosine from 4±1 [7] to 6±1 [5] percent ($p<0.05$). Theophylline (1 mM) did not increase the recovery of adenosine.

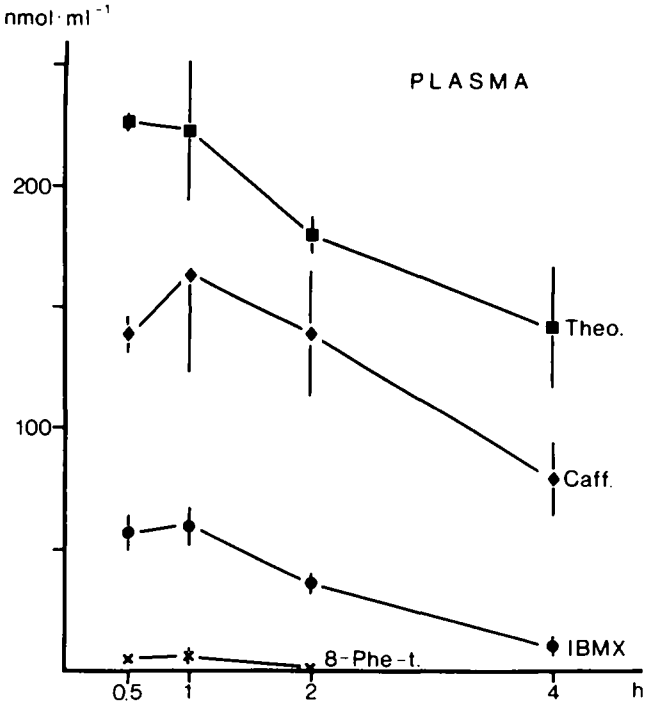

FIG. 2. Concentration of theophylline (theo), caffeine (caff), isobutylmethylxanthine (IBMX) and 8-phenyl-theophylline (8Phe-t) in rat plasma at different times after IP administration after administration of 20, 20, 5 and 10 mg/kg, respectively, at 0-time. No significant levels (below 1 nmol/ml) of either xanthine was obtained in 0-time samples. The first three drugs were dissolved in saline, while the last was dissolved in 0.03% tetraphenyl-boronate in saline. Mean±S.D. of 3–5 determinations.

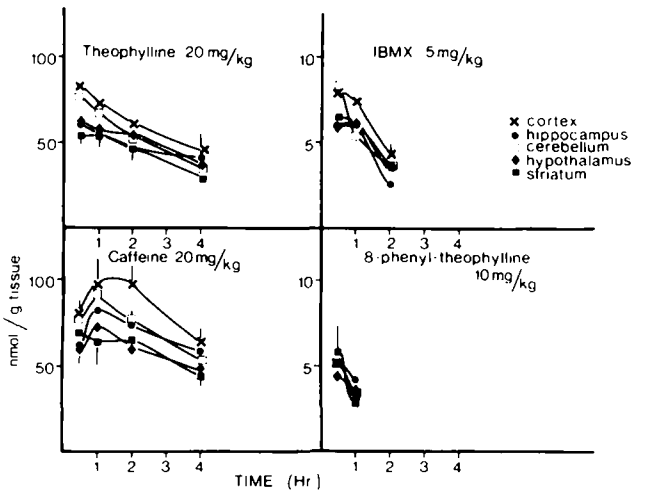

FIG. 3. Regional distribution of alkylxanthines in the rat brain. x—cortex, ●—hippocampus, ◆—hypothalamus, ■—striatum, ❑—cerebellum. For details see text and legend to Fig. 2. Means; For graphical reasons s.e.m. is indicated only for some points, but there were no major differences between regions or in the intraregion variability. Theophylline, n=4; caffeine, n=4; IBMX, n=3; 8-phenyl-theophylline, n=3.

Metabolism of labelled adenosine to inosine, hypoxanthine and uric acid was inhibited by 1 μM EHNA. The ratio of labelled adenosine metabolites to labelled adenosine was 18±4 percent in control perfusions and 11±3 percent in the presence of 1 μM EHNA ($p<0.05$). Such inhibition was seen irrespective of whether labelled ATP, AMP or adenosine was superfused over the slices.

Levels of Methylxanthines in Brain

In order to be able to determine the potencies of methylxanthines in brain it was essential to determine their brain concentration. For comparison the concentrations in plasma were determined. The concentration in plasma of caffeine, theophylline, isobutyl-methylxanthine and 8-phenyl-theophylline given in the amounts 20, 20, 5 and 10 mg/kg, IP, respectively, are shown in Fig. 2. The regional distribution in the brain is shown in Fig. 3. Since the levels in plasma and brain are similar no attempt was made to correct for the contribution by regional blood content to the measured brain levels (less than 10 percent). For 8-phenyl-theophylline there were no clear regional differences. Furthermore, the levels after more than 1 hour could not be determined with any certainty as the levels were at the detection-limit (close to 2 nmol/μg protein). 8-Phenyl-theophylline was not significantly broken down to theophylline. Samples of epididymal adipose tissue tended to show large peaks, suggesting that the drug might accumulate in fat, but adipose tissue contains interfering materials with a chromatographic behaviour similar to that of 8-phenyl-theophylline, making quantitative estimates of the amounts deposited in fat difficult. The levels of 8-phenyl-theophylline fell rapidly between 0.5 and 1 hour (Fig. 3). Also for theophylline and IBMX the level in brain was highest at 0.5 hour after administration, while caffeine levels peaked between 1 and 2 hours after administration. For all three methylxanthines the highest levels were found in cerebral cortex, while the levels in other regions were 60–85 percent of those in cerebral cortex. The levels in striatum were only $^2/_3$ of that in cerebral cortex. Metabolism of caffeine to theophylline (or paraxanthine) was not important since in only 6 samples (of 80) could theophylline peaks higher than 5 percent of the caffine peak in the same sample be detected. The ratio between amounts in plasma and cerebral cortex (at 1 hour) was lowest for caffeine (1.65) followed by 8-phenyl-theophylline (1.7), theophylline (3.1) and IBMX (7.8 ml g^{-1}). The relative brain concentrations of caffeine, theophylline and IBMX agree well with that reported by Snyder *et al.* [32] for mouse brain. Similarly the distribution of caffeine between plasma and brain agrees well with that reported by Latni *et al.* [24].

Rotation Behaviour

Actions of apomorphine alone. The two-peak pattern of rotation noticed previously [35] in successfully 6-OHDA denervated rats is shown in Figs. 4 and 5. It is also seen that within each group of animals the extent of this rotation was very reproducible. On the average, apomorphine (0.05 mg/kg) caused a total rotation amounting to 715+18 turns (n=14). The total duration of the rotation averaged 76±3 min in the same rats.

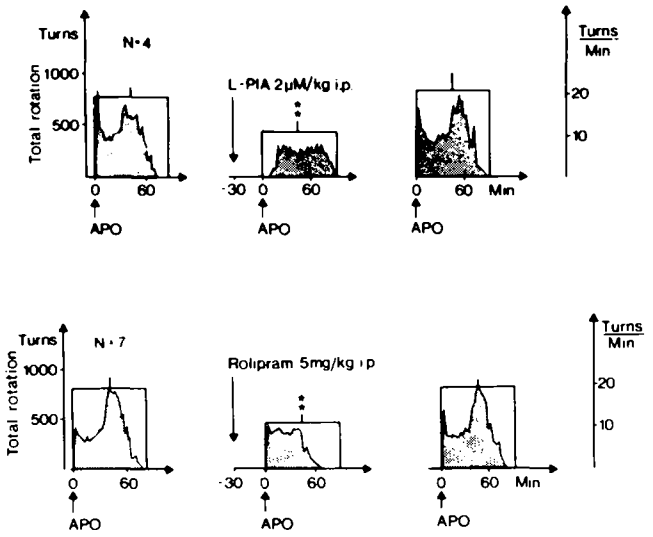

FIG. 4. The effect of L-PIA 2 μmol/IP and rolipram 5 mg/kg (IP) on the rotation induced by apomorphine 0.05 mg/kg. The shaded diagram shows the mean of the individual rotometer recordings in 7 animals (turns/min on the ordinate; time on the abscissae). The bar graph shows the total number of turns and the s.e.m. (ordinate—total No. of turns). For experimental details see Methods section. ★★ represents a significant difference in total number of turns ($p<0.01$).

Actions of theophylline. In agreement with previous reports [11, 17, 19, 37] we found that theophylline per se caused a dose-dependent stimulation of rotation behaviour (Table 1). This rotation is of long duration, relatively low intensity and shows characteristics that makes it different from both apomorphine and ergot-type dopamine receptor agonists [17, 18, 37]. As shown in Table 1, pretreatment with theophylline enhanced the total number of turns induced by apomorphine. At the higher dose used, the enhancement could be ascribed mainly to the addition of a potent effect of theophylline. At the lower dose (10 mg/kg) the observed 30±26 percent enhancement (based on percental changes in each individual experiment, $p<0.05$) could not be entirely explained by a stimulatory effect of theophylline alone (Table 1). As shown in Fig. 5 the rotation induced by apomorphine + theophylline was much larger than that due to apomorphine alone. We have also in that figure illustrated the effect of theophylline alone. The experiments were, however, not designed in such a way that the effect of apomorphine on top of theophylline could be evaluated statistically. The results given in Table 1 are from separate experiments using separate rats.

Action of rolipram. The potent cyclic AMP phosphodiesterase inhibitor rolipram significantly depressed the apomorphine induced rotation behaviour. At a dose of 1 mg/kg a 34±8 percent inhibition was found and at 5 mg/kg a 39±9 percent inhibition. The effect of 5 mg/kg rolipram is shown in Fig. 4.

Action of 8-phenyl-theophylline. 8-Phenyl-theophylline, which is a highly potent adenosine antagonist that is almost devoid of phosphodiesterase inhibitory effect [7,31], caused a slight enhancement of the rotation induced by apomorphine. At 5 mg/kg the apomorphine response was 108±8, at

TABLE 1

ROTATION BEHAVIOUR INDUCED BY APOMORPHINE AND THEOPHYLLINE, ALONE AND IN COMBINATION

Drug	Dose (mg/kg)	Total turns
Apomorphine	0.05	715 ± 18 (24)
Theophylline	10	95 ± 21 (8)
Theophylline	25	1296 ± 198 (8)
Apomorphine	0.05	
+ Theophylline	10	896 ± 66 (4)
+ Theophylline	25	1595 ± 198 (4)

Mean ± s.e.m. Number of experimental animals within parenthesis.

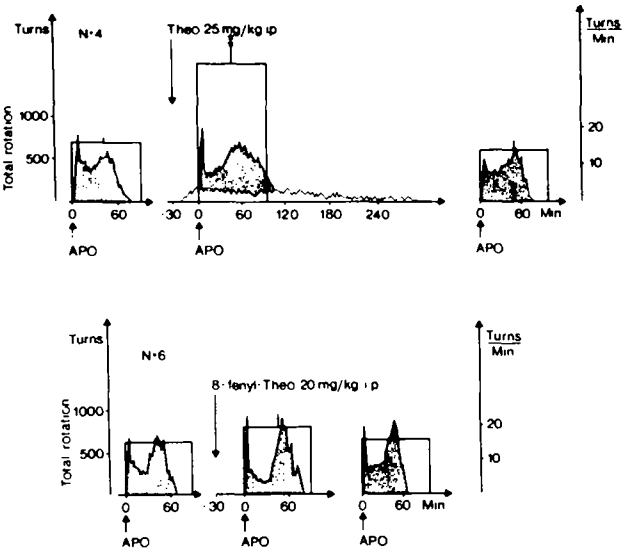

FIG. 5. The effect of theophylline 25 mg/kg (IP) and 8-phenyl-theophylline 20 mg/kg (IP) on apomorphine induced rotation behaviour. The effect of theophylline alone is shown by the open curve. The shaded area thus represents the effect of apomorphine after theophylline. For explanations see legend to Fig. 4.

10 mg/kg it was 127±15 and at 20 mg/kg 133±19 percent of control ($p<0.05$). The effect of the highest dose is shown in Fig. 5. It is seen that 8-phenyl-theophylline tended to enhance the trough between the two peaks and to enhance the second peak.

Actions of L-PIA and EHNA. L-PIA given alone tended to produce profound muscle relaxation in the animals, but they were not asleep. This is in agreement with previous results in both rats [38] and mice [32]. It caused a potent, dose-dependent inhibition of apomorphine-induced rotation. At a dose of 0.2 μmol/kg it inhibited the rotation by 30±4, at 2 μmol/kg by 45±10 and at 5 μmol/kg by 55±12 percent (n=4). The response to 2 μmol/kg is shown in Fig. 4. It is seen that L-PIA abolishes the first peak of rotation completely and considerably blunts the second peak.

The adenosine deaminase inhibitor EHNA had small effects. At 2 mg/kg it reduced the rotation by 32±5 percent.

Five mg/kg, by contrast, was essentially ineffective (15±11 percent).

DISCUSSION

The present study has provided further support for the notion that inhibition of cyclic AMP hydrolysis is not the mechanism by which methylxanthines such as theophylline and caffeine enhance the actions of dopamine receptor agonists. Thus, the selective cyclic AMP phosphodiesterase inhibitor rolipram reduced rather than enhanced rotation. This is in agreement with earlier results of Arbuthnott *et al.* [1] using some other non-methylxanthine phosphodiesterase inhibitors. When considered together with other evidence alluded to in the introduction this finding strongly indicates that the mechanism behind the methylxanthine effect must be sought elsewhere.

The present results tend to support the previously made suggestion that endogenous adenosine may inhibit rotation behaviour [9]. Thus, L-PIA which is a potent agonist at adenosine receptor of the A1-subtype [5, 26, 32] caused a marked behavioural depression. Very recently Green and coworkers demonstrated that the intrastrial injection of an adenosine analogue caused inhibition of apomorphine actions in that striatum, leading to a rotation behaviour [20]. The levels of endogenous adenosine in rat brain are high enough to cause activation of adenosine receptors [39]. EHNA caused only a slight reduction of the rotation behaviour in agreement with the finding that it causes only limited changes in the concentration of free diffusible adenosine in the brain [38]. 8-Phenyl-theophylline, which is quite selective as an antagonist of adenosine action [30], could mimic the actions of theophylline. As a competitor for adenosine binding sites [5], as an antagonist of adenosine actions on cyclic AMP in hippocampal slices [13] on adenosine action in fat cells [24] and on adenosine action on interictal spike activity in hippocampus [7] 8-phenyl-theophylline was some 30–60 times more potent than theophylline. In the present study 10 mg/kg 8-phenyl-theophylline gave an effect that was approximately equivalent with that observed with 25 mg/kg theophylline. The pharmacokinetic results shown in Fig. 3 indicate that the brain levels of 8-phenyl-theophylline were 10–20 times lower than those of theophylline at an equivalent dose. The plasma levels may be some 20 times lower. Hence, our results suggest that 8-phenyl-theophylline may be 25–50 times more potent than theophylline, in good agreement with the above mentioned relative potencies in different *in vitro* systems.

Theophylline and caffeine are known to produce rotation behaviour per se. 8-Phenyl-theophylline per se caused little or no stimulation of rotation behaviour on the other hand. The explanation does not seem to reside in the fact that 8-phenyl-theophylline is a poorer inhibitor of cyclic AMP hydrolysis [30] since selective inhibitors of the enzyme caused no stimulation of rotation behaviour ([1] and present results). On the other hand, level of 8-phenyl-theophylline in brain may have been insufficient and the duration of its presence in the brain may have been too short to have produced any stimulation per se. The present and earlier results [19,36] suggest that potentiation of apomorphine-induced rotation may require lower doses than does induction of rotation behaviour by the methylxanthine alone. The findings of Snyder *et al.* [31] in mice suggest a relationship between potency of various alkylxanthines as adenosine receptor antagonists and as stimulators of motor behaviour in mice. There are several possible ways that adenosine may modulate rotation behaviour and hence that methylxanthines could exert their action via adenosine mechanisms. First of all adenosine may interfere with the release dopamine and other transmitters that are of importance in this behaviour [9]. Secondly, there may be a direct inhibitory effect of adenosine on dopamine receptor mediated effects in the striatum [28]. Finally, adenosine may cause a general behavioural depression that reduces i.a. rotation behaviour (cf., [1]). The present results do not lend themselves well to a clarification of which of these potential mechanisms is the more important. Furthermore, the possibility also exists that stimulation of motor behaviour by caffeine and theophylline is unrelated to either phosphodiesterase inhibition or adenosine receptor antagonism and that it is due to some as yet uncharacterized mechanism of action.

ACKNOWLEDGEMENTS

We are grateful to Mr. Dan Olsson and Mrs. Louise Vernet for skillful technical assistance. The present work was supported by the Swedish Medical Research Council (project no. 2553 and 3574), by Wibergs Foundation, Ostermans Foundation and M. Bergvalls Foundation.

REFERENCES

1. Arbuthnott, G. W., T. J. Attree, D. Eccleston, R. W. Loose and M. J. Martin. Is adenylade cyclase the dopamine receptor? *Med Biol* **52**: 350–359, 1974.
2. Arch, J. R. S. and E. A. Newsholme. The control of the metabolism and the hormonal role of adenosine. In: *Essays in Biochemistry*, vol 14, edited by P. W. Campbel and W. N. Aldridge. London: Academic Press, 1978, pp. 88–123.
3. Boissier, J. R. and P. Simon. Action de la caféine sur la motilitée spontanée de la souris. *Arch Int Pharmacodyn Ther* **158**: 212–221, 1965.
4. Brown, B. L., R. P. Ekins and J. D. M. Albano. Saturation assay for cyclic AMP using endogenous binding protein. *Adv Cyclic Nucleotide Res* **2**: 25–40, 1972.
5. Bruns, R. F., J. W. Daly and S. H. Snyder. Adenosine receptors in brain membranes: Binding of N^6-cyclo-hexyl-[^{3}H]adenosine and 1,3-diethyl-8-[^{3}H]phenylcanthine. *Proc Natl Acad Sci USA* **77**: 5547–5551, 1980.
6. Butcher, R. W. and E. W. Sutherland. Adenosine 3',5'-phosphate in biological materials. I. *Biol Chem* **237**: 1244–1250, 1962.
7. Dunwiddie, T. V., B. J. Hoffer and B. B. Fredholm. Alkylxanthines elevated hippocampal excitability: Evidence for a role of endogenous adenosine. *Naunyn Schmiedebergs Arch Pharmacol* **316**: 326–330, 1981.
8. Fox, I. H. and W. N. Kelley. The role of adenosine and 2-deoxy-adenosine in mammalian cells. *Annu Rev Biochem* **47**: 655–688, 1978.
9. Fredholm, B. B. Dopamine receptors and phosphodiesterases. In: *Dopaminergic Ergot Derivatives and Motor Function*, edited by D. B. Calne and K. Fuxe. New York: Pergamon Press, 1979, pp. 73–83.
10. Fredholm, B. B. Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends Pharmacol Sci* **1**: 129–132, 1980.

11. Fredholm, B. B., K. Fuxe and L. Agnati. Effect of some phosphodiesterase inhibitors on central dopamine mechanisms. *Eur J Pharmacol* **38:** 31–38, 1976.
12. Fredholm, B. B., M. Herrera-Marschits and U. Ungerstedt. Rotation behaviour induced by methylxanthines: Phosphodiesterase inhibition, adenosine antagonism or some unknown mechanism of action? *Acta Pharmacol Toxicol* **49:** Suppl III 16–17, 1981.
13. Fredholm, B. B., B. Jonzon, E. Lindgren and K. Lindström Adenosine receptors mediating cyclic AMP production in the rat hippocampus. *J Neurochem* **39:** 165–175, 1982.
14. Fredholm, B. B. and A. Sollevi. The release of adenosine and inosine from canine subcutaneous adipose tissue by nerve stimulation and noradrenaline. *J Physiol* **313:** 351–367, 1981.
15. Fredholm, B. B. and L. Vernet. Release of ^{3}H-nucleosides from ^{3}H-adenine labelled hypothalamic synaptosomes. *Acta Physiol Scand* **106:** 97–197, 1979.
16. Fredholm, B. B., L. Vernet, B. Jonzon, M. Herrera-Marschitz, U. Ungerstedt and T. Zetterström. Antagonism of endogenous adenosine—a possible cause of theophylline induced rotation behaviour. *Prog Neuropsychopharmacol* Suppl. 147, 1980.
17. Fuxe, K., B. B. Fredholm, L. F. Agnati, S.-O. Ögren, B. J. Everitt, G. Jonsson and J.-Å. Gustafsson. Interaction of ergot Drugs with central monoamine systems. Evidence for a high potential in the treatment of mental and neurological disorders. *Pharmacology* **16:** Suppl 1, 99–134, 1978.
18. Fuxe, K., B. B. Fredholm, S.-O. Ögren, L. F. Agnati, T. Hökfelt and J.-Å. Gustafsson. Pharmacological and biochemical evidence for the dopamine agonistic effect of bromocriptine. *Acta Endocrinol* (Copenh) **88:** Suppl 216, 27–56, 1978.
19. Fuxe, K. and U. Ungerstedt. Action of caffeine and theophyllamine on supersensitive dopamine receptors: considerable enhancement of receptor response to treatment with dopa and dopamine receptor agonists. *Med Biol* **52:** 48–54, 1974.
20. Green, R. D., H. K. Proudfit and S.-M. H. Young. Modulation of striatal dopaminergic function by local injection of 5′-N′-carboximideadenosine. *Science* **218:** 58–61, 1982.
21. Herz, A., B. Neteler and H. J. Teschemacher. Vergleichende untersuchungen über zentrale Wirkungen von Xanthinderivaten in Hinblick auf deren Stoffwechsel und Verteilung im Organismus. *Naunyn Schmiedebergs Arch Pharmacol* **261:** 486–502, 1968.
22. Kebabian, J. W., G. L. Petzold and P. Greengard. Dopamine-sensitive adenylate cyclase in the caudate nucleus of rat brain, and its similarity to the dopamine receptor. *Proc Natl Acad Sci USA* **69:** 2145–2149, 1972.
23. König, J. and R. C. Klippel. *The Rat Brain: A Stereotaxic Atlas*. New York: R. E. Krieger, 1963.
24. Latini, R., M. Bonati, E. Marji, M. T. Tacconi, B. Sadurska and A. Bizzi. Caffeine disposition in young and one-year old rats. *J Pharm Pharmacol* **32:** 596–598, 1980.
25. Lindgren, E. and B. B. Fredholm. Effect of different alkylxanthines on adenosine-receptor mediated inhibition of fat cell cyclic AMP accumulation. *Acta Pharmacol Toxicol* **49:** Suppl III, 29, 1981.
26. Londos, C., D. M. F. Cooper and J. Wolff. Subclasses of external adenosine receptors. *Proc Natl Acad Sci USA* **77:** 2551–2554, 1980.
27. Satoh, H., Y. Satoh, Y. Notsu and F. Honda. Adenosine 3′,5′-cyclic-monophosphate as a possible mediator of rotational behaviour induced by dopaminergic receptor stimulation in rats lesioned unilaterally in the substantia nigra. *Eur J Pharmacol* **39:** 365–377, 1976.
28. Schaeffer, H. J. and C. F. Schwender. Enzyme inhibitors. 26. Bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl)adenines. *J Med Chem* **17:** 6–8, 1974.
29. Schwabe, U., M. Miyake, Y. Ohga and J. W. Daly. 4-(3-Cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (ZK 62, 711): A potent inhibitor of cyclic AMP phosphodiesterases in homogenates and tissue slices from rat brain. *Mol Pharmacol* **12:** 900–910, 1976.
30. Schwabe, U. and T. Trost. Characterization of adenosine receptors in rat brain by (−) (^{3}H)N^6-phenylisopropyl-adenosine. *Naunyn Schmiedebergs Arch Pharmacol* **313:** 179–187, 1980.
31. Smellie, F. W., C. W. Davis, J. W. Daly and J. N. Wells. Alkylxanthines: Inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. *Life Sci* **24:** 2475–2482, 1979.
32. Snyder, S. H., J. J. Katims, Z. Annau, R. F. Bruns and J. W. Daly. Adenosine receptors and behavioural actions of methylxanthines. *Proc Natl Acad Sci USA* **78:** 3260–3264, 1981.
33. Thithapandha, A., H. M. Mailing and J. R. Gillette. Effects of caffeine and theophylline on activity of rats in relation to brain xanthine concentrations. *Proc Exp Biol Med* **139:** 582–586, 1972.
34. Ungerstedt, U. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxy-dopamine on monoamine neurons in the rat brain. In: *6-Hydroxy-Dopamine and Catecholamine Neurons*, edited by T. Malmfors and H. Thoenen. Amsterdam: North-Holland, 1971, pp. 101–127.
35. Ungerstedt, U. and G. Arbuthnott. Quantitative recording of rotational behaviour in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res* **24:** 485–493, 1970.
36. Ungerstedt, U. and M. Herrera-Marschitz. Behavioural pharmacology of dopamine receptor mechanisms. In: *Chemical Neurotransmission: 75 years*, edited by L. Stjärne, H. Lagercrantz, P. Hedqvist and Å. Wennmalm. New York: Academic Press, 1981, pp. 481–494.
37. Ungerstedt, U., M. Herrera-Marschitz and M. C. Brugue. Are apomorphine bromocriptine and the methylxanthines agonists at the same dopamine receptors? In: *Apomorphine and Other Dopaminomimetics, vol 1: Basic Pharmacology*, edited by G. L. Gessa and G. U. Corsini. New York: Raven Press, 1981, pp. 85–93.
38. Vapaatalo, H., D. Onken, P. Neuronen and E. Westerman. Stereospecificity in some central and circulatory effects of phenylisopropyladenosine. *Arzneimittleforsch* **25:** 407–410, 1975.
39. Zetterström, T., L. Vernet, U. Ungerstedt, U. Tossman, B. Jonzon and B. B. Fredholm. Purine levels in the intact brain. Studies with an implanted perfused hollow fibre. *Neurosci Lett* **29:** 111–115, 1982.