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Brain Distribution Characteristics of Xanthine Derivatives and Relation to their Locomotor Activity in Mice

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Abstract

The relationship between the brain distribution and motor activity in mice of the xanthines, theophylline, enprofylline, 1-methyl-3-propylxanthine (MPX) and oxpentifylline was investigated. Their plasma protein binding and hydrophobicity were also examined.

When these xanthines were administered orally, enprofylline and oxpentifylline had no effect on motor activity. While theophylline increased motor activity over 10 mg kg^{-1}, MPX caused a decrease in such activity over 10 mg kg^{-1}. The protein-binding behaviour varied among these xanthines and was closely related to their hydrophobicity, which is represented as a logarithmic partition coefficient (log PC). MPX had the highest hydrophobicity, while oxpentifylline had the lowest. Brain distribution characteristics varied among these xanthines, with the rank order of their brain penetration ratio, calculated as the ratio of brain to unbound plasma concentrations, being theophylline > oxpentifylline > MPX > enprofylline. The inhibition constants (K_i) for adenosine A_1 receptors and cyclic 3′,5′-adenosine monophosphate (cAMP)-phosphodiesterase (PDE) of these xanthines were 44·6 and 134, > 1000 and 112, 26·4 and 49, and > 1000 and 111 μM for theophylline, enprofylline, MPX, and oxpentifylline, respectively.

These findings suggest that the lack of effects of enprofylline and oxpentifylline on motor activity is probably due to their low brain penetration ratio or low adenosine A_1 affinity in comparison with theophylline. The decrease in the motor activity by MPX may be, in part, mediated by cAMP or adenosine.

Many reports on the pharmacokinetics and pharmacological activities of xanthines have been published. A number of hypotheses for the mechanism of their pharmacological activities from cyclic 3′ 5′-adenosine monophosphate (cAMP)-phosphodiesterase (PDE) inhibition and adenosine antagonism have been suggested. Cortijo et al (1993) recently found a direct relationship between bronchodilatory and cAMP-PDE inhibitory activities of theophylline and some other xanthines while the same relationship has also been obtained for either PDE inhibitory activity or affinity for adenosine A_1 receptors of xanthines in our laboratories (Takagi et al 1992). It is thought that xanthines have a bronchodilatory effect through both inhibition of cAMP-PDE and adenosine-receptor antagonism, and that most side-effects produced by the xanthines are due to the antagonism of central and cardiac adenosine A_1 receptors (Daly 1985; Persson et al 1986). However, the precise mechanism for xanthine-induced bronchodilatory effect and side-effects has not yet been clarified. Our group has synthesized various xanthine derivatives in order to design more effective anti-asthmatic drugs without side-effects. We have demonstrated a close relationship between their smooth relaxant and cAMP-PDE inhibitory activities and that a newly-developed xanthine, 1-methyl-3-propylxanthine (MPX), has a more potent relaxant effect in-vitro and in-vivo than has either theophylline or enprofylline (Apichartpichean et al 1988; Ogawa et al 1989; Miyamoto et al 1989, 1993; Hasegawa et al 1990a, 1991b; Sakai et al 1992; Takagi et al 1992). Enprofylline is not an adenosine antagonist, but is a potent cAMP-PDE inhibitor (Persson & Kjellin 1981; Lunell et al 1982, 1983). However, no significant data on the side-effects of either enprofylline or MPX are available. Although oxpentifylline has been reported to have bronchorelaxation ability in human bronchial tissue in-vitro and in guinea-pigs in-vitro and in-vivo (Cortijo et al 1993), it is a vasodilator which is clinically used for patients with various circulatory disorders, including those in the brain. It is therefore to be expected that it will penetrate well into the brain and have a low affinity for adenosine receptors, as has indeed been reported by Hand et al (1989). Our recent studies demonstrated that the hydrophobicity of xanthines plays an important role in their cAMP-PDE inhibitory activity and affinity for adenosine A_1 receptors, and that their protein binding potency to serum albumin is closely related to their hydrophobicity, thus constituting an important determinant for their biological activities (Hasegawa et al 1990a, 1991b; Takagi et al 1992). These observations suggest that the protein-binding and hydrophobicity of xanthine bronchodilators are important keys for the elucidation of their pharmacological activity. The present study aims firstly to determine the brain distribution characteristics of the four xanthines selected, theophylline, enprofylline, MPX and oxpentifylline (Fig. 1), after oral administration in mice, and secondly to evaluate the relationship between plasma

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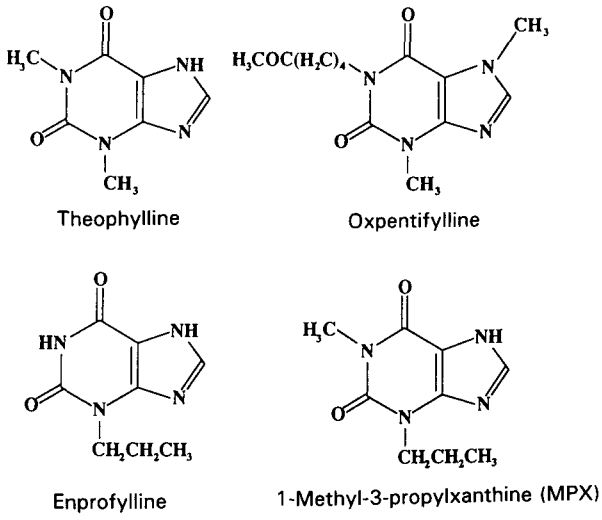

FIG. 1. Chemical structures of xanthine derivatives used in this study.

and brain levels of these xanthines and their motor activity, and the possibility of their relation to affinity for adenosine A_1 receptor. The roles of their protein-binding and hydrophobicity in the brain distribution and the relationship between their brain distribution and cAMP-PDE inhibitory activity or affinity for adenosine A_1 receptors were also investigated.

Materials and Methods

Materials

The *N*-alkylxanthine derivatives, enprofylline, oxpentifylline, 1-methyl-3-propylxanthine (MPX), 3-butylxanthine and 1-methyl-3-butylxanthine, were synthesized in our laboratory and were identical to those previously reported (Apichartpichean et al 1988, 1991; Takagi et al 1988; Hasegawa et al 1990a, b, 1991a, b; Nadai et al 1991, 1993; Muraoka et al 1992; Miyamoto et al 1993). Theophylline was obtained from Sigma Chemical Co. (St Louis, MO, USA). [^{3}H]cAMP and [^{3}H]1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]CPX) (New England Nuclear, Boston, MA, USA) were used to measure PDE activity and adenosine-receptor-blocking activity, respectively. All other chemicals were commercially available and used without further purification. For in-vivo experiments, enprofylline and MPX were suspended in isotonic saline and sodium hydroxide was added in drops to make a clear solution. Theophylline and oxpentifylline were dissolved in isotonic saline. For in-vitro experiments, all tested compounds were prepared in the respective assay buffer.

Animal experiments

Male ddY mice (Nippon SLC, Hamamatsu, Japan), weighing 22–32 g, were used in this study. The mice were housed in a temperature (22–24°C) and light-controlled (light on 0900 to 2100 h) room with commercial food and water freely available. Theophylline (10 mg kg^{-1}), enprofylline (30 mg kg^{-1}) MPX (3 and 10 mg kg^{-1}) and oxpentifylline (100 mg kg^{-1}) were administered orally. Five mice for each drug dose were killed at the designated intervals (10, 20, 30, 45 and 60 min) after administration. Blood was collected from the abdominal vein and the mice were then decapitated. Plasma, obtained by centrifugation, and brain samples were stored at −30°C until required for analysis. Biological activities were measured using brain matter from mice killed by cervical dislocation followed by exsanguination. The brain was rapidly removed.

Measurement of spontaneous motor activity

Spontaneous motor activity was measured individually in a rodent housing cage using an Animex AUTO MK-110 (Muromachi Kikai, Tokyo, Japan) for 60 min after oral administration of each compound.

High-performance liquid chromatography (HPLC) assay

HPLC conditions for each compound in blood and brain samples were investigated using blank plasma and brain; there was no interference at the retention time of either compound. The mobile phase was 30 mM phosphate (KH_2PO_4) buffer with different pH values. Standard curves were constructed over a range of 1·11–66·6 μM for theophylline, 2·58–257 μM for enprofylline, 0·18–35·9 μM for oxpentifylline, and 2·40–192 μM for MPX in plasma, and 0·056–2·22 μM for theophylline, 2·58–51·5 μM for enprofylline, 0·18–7·19 μM for oxpentifylline and 0·48–120 μM for MPX in brain matter. These standard curves were linear in all cases. In all cases recoveries for compounds were > 95% with coefficients of variation < 5%. The conditions for each compound and the appropriate internal standard are given in Table 1.

Fifty microlitres of plasma was deproteinized by adding 350 μL methanol containing an internal standard and centrifuged at 6000 *g* for 5 min. The resulting supernatant was evaporated by means of a gentle stream of N_2 gas at 40°C. The residue was reconstituted in 200 μL mobile phase and was injected into the HPLC. The apparatus was a Shimadzu LC-6A system (Shimadzu Company, Kyoto, Japan) consisting of an LC-6A liquid pump, an SPD-6A

Table 1. HPLC conditions for plasma and brain.

Xanthine	Mobile phase (buffer/methanol)		pH of buffer		Column temp. (°C)		Internal standard
	Plasma	Brain	Plasma	Brain	Plasma	Brain	
Theophylline	91/9	91/9	3	3	40	40	Enprofylline
Enprofylline	80/20	85/15	5	3	50	40	3-Butylxanthine
MPX	74/26	75/25	3	3	50	40	1-Methyl-3-butylxanthine
Oxpentifylline	68/32	66/34	7	5	35	35	1-Methyl-3-butylxanthine

UV spectrophotometric detector, and a SIL-6A auto-injector. The UV detector was set at 274 nm and a Cosmosil 5C18 column (Nacalai Tesque, Kyoto, Japan) was used.

Whole brain was homogenized in 10 mL saline in a volumetric flask with an Astrason Ultrasonic Processor XL (Wakenyaku Co. Ltd, Tokyo, Japan). One millilitre of homogenate and 1 mL 1 M HCl as aqueous phase were added to 5 mL ethyl acetate as organic phase, shaken for 10 min and then centrifuged. The organic phase was evaporated by means of a gentle stream of N_2 gas at 50°C. The residue was reconstituted in 300 μL mobile phase and was injected into the HPLC. A preliminary experiment had shown that ethyl acetate is the best extractive organic solvent and that the optimum pH was 3.

Protein-binding experiments

The binding of each compound to plasma protein was measured by the micropartition equilibrium dialysis method using cellulose membrane (Visking sheet, Sanplatec Corp., Osaka, Japan) with a molecular weight cut-off set at 10 000–20 000. Blood samples were obtained from 12 control mice by exsanguination from the abdominal aorta under light ether anaesthesia; plasma samples were obtained immediately by centrifugation. Freshly prepared plasma (0·4 mL) was dialysed against an equal volume of isotonic phosphate buffer (pH 7·4) containing the desired concentrations of compounds (14·4 and 48·0 μM for MPX, 10·8 μM for oxpentifylline, 55·5 μM for theophylline and 103 μM for enprofylline) at 37°C for 5 h to attain equilibrium, as described elsewhere (Apichartpichean et al 1991; Muraoka et al 1992; Nadai et al 1993). After dialysis the concentrations of each compound in both sides of the membrane were measured by HPLC.

Measurement of cAMP-PDE

Cerebral cortex was homogenized in a Tris HCl buffer (composition in mM: Tris 40, $MgCl_2$ 10, 2-mercaptoethanol 4; pH 8·0) and then centrifuged at 10 000 *g*. cAMP-PDE activity (Ca^{2+}/calmodulin independent) with low K_m of 1·17 μM and V_{max} of 303·0 pmol min^{-1} (mg protein)$^{-1}$ in the supernatant was measured by the two-step assay system of Thompson & Appleman (1971). In the present study 1 mM glycolethylenediamine-*N*-tetraacetic acid (GEDTA) was added to a reaction mixture containing the test compound or vehicle, cAMP and [^{3}H]cAMP to remove calmodulin-dependent cAMP-PDE. The inhibition constant (K_i) of each compound was calculated by the method of Dixon (1953).

Measurement of adenosine-receptor binding

Binding to the mouse brain membrane preparation and ligand binding assay were performed essentially as described by Jacobson et al (1986). A selective antagonist of the adenosine A_1 receptor [^{3}H]CPX was used in this study. The Scatchard plots of CPX binding to the brain membrane were linear with a K_d value of 3·97 nM and a B_{max} value of 290·1 fmol (mg protein)$^{-1}$. A mixture of 2·0 nM [^{3}H]CPX, various concentrations of each compound, 0·2 units of adenosine deaminase and approximately 100 μg protein of the membrane preparation in 50 mM Tris HCl buffer (pH 7·4) were incubated at 37°C for 120 min to conduct an examination of the ligand-binding replacement. Bound and unbound radioligands were separated by rapid filtration through Whatman GF/B glass-fibre filters using a cell harvester (Model 2000 PHD, Cambridge Technology, Cambridge, MA). The filters were treated with 0·3% polyethyleneimine before use. The nonspecific binding of [^{3}H]CPX to the membrane was measured in the presence of 10 mM theophylline. Dose-inhibition data for each compound were analysed using a nonlinear least-squares fitted to a competitive-inhibition model. The inhibition constant (K_i) was calculated from the Cheng-Prusoff equation (Cheng & Prusoff 1973). Protein was measured by the method of Lowry et al (1951).

Measurement of apparent partition coefficient

Partition coefficients of each compound were measured by a procedure described previously (Hasegawa et al 1990a). Briefly 4 mL pH 7·4 isotonic phosphate-buffer solution containing the desired concentration of each compound (55·5, 155, 10·8 and 24·0 μM for theophylline, enprofylline, oxpentifylline and MPX, respectively) was added to an equal volume of *n*-octanol, and equilibrated by continuous shaking at 37°C for 4 h. The concentrations of each compound in the organic and aqueous phases were determined by spectrophotometry at 274 nm. The apparent partition coefficient (PC) of each compound was calculated as the ratio of the concentration in the organic phase to that in the aqueous phase, and the hydrophobicity was expressed as a logarithmic partition coefficient (log PC).

Statistical analysis

The results were expressed as mean ± s.e.m. The regression lines were constructed using linear regression analysis (Yamaoka et al 1981).

Results

Motor activity in mice during the 60 min following a single oral administration of different doses of these xanthine derivatives is shown in Table 2. The motor activity of theophylline at a dose of 1 mg kg^{-1} showed no changes from the control, but increased with increasing the dose (3 and 10 mg kg^{-1}), in agreement with the reported data that theophylline increases general motor activity in the range 3–10 mg kg^{-1} (Stahle et al 1990). On the other hand, MPX at a dose of 10 mg kg^{-1} decreased the motor activity by

Table 2. Effects of xanthines on motor activity in mice.

Experiment	Dose (mg kg^{-1})	Locomotor counts (mean ± s.e.m.)
Control (n = 15)		1430 ± 115
Theophylline (n = 8)	1	1460 ± 146
	3	1780 ± 186
	10	1950 ± 140**
MPX (n = 8)	3	1470 ± 210
	10	660 ± 130**
Enprofylline (n = 9)	10	1600 ± 85
	30	1360 ± 115
Oxpentifylline (n = 8)	30	1430 ± 130
	100	1150 ± 85

**$P < 0·05$ compared with control.

Table 3. Physicochemical properties of xanthines.

Xanthine	Unbound fraction (fu)	Apparent partition coefficient (log PC)
Theophylline	0·90 ± 0·03	0·072 ± 0·011
Enprofylline	0·73 ± 0·02	0·383 ± 0·002
MPX	0·41 ± 0·06	0·951 ± 0·017
Oxpentifylline	0·95 ± 0·11	−0·024 ± 0·020

Each value represents mean ± s.e.m. of three determinations.

approximately 50%. No significant changes in the motor activity for enprofylline or oxpentifylline were observed. Table 3 shows the physicochemical properties of the unbound fraction of each xanthine (fu) and apparent partition coefficient (log PC) which is represented as hydrophobicity. Marked differences in protein-binding potency and hydrophobicity were observed among these xanthine derivatives. The binding potency to mouse plasma protein for enprofylline and MPX was relatively low (< 60%) which was consistent with our previous studies (Tsunekawa et al 1992). The rank order of hydrophobicity was MPX > enprofylline > theophylline > oxpentifylline, which was again consistent with our previous studies (Hasegawa et al 1990a).

Fig. 2 shows the mean semilogarithmic plots of total and unbound plasma concentration–time data for each xanthine after a single oral administration of theophylline (10 mg kg^{-1}), enprofylline (30 mg kg^{-1}), MPX (3 and 10 mg kg^{-1}) and oxpentifylline (100 mg kg^{-1}) to mice. The slight differences between total and unbound plasma concentrations of theophylline and oxpentifylline may be explained by their low protein-binding potency. However, significant differences in the corresponding curves for MPX and enprofylline can be observed (Fig. 2). Fig. 3 shows the brain concentration–time curves for each drug. The disappearance of theophylline from plasma and brain seems to be the most delayed in comparison with the others. Maximum concentrations in both plasma and brain were reached at the first measurement (10 min) for oxpentifylline, at 20 min for MPX and theophylline, and at 30 min for enprofylline. The distribution of oxpentifylline into the brain and its plasma disappearance were rapid.

As the disappearance of xanthine derivatives from plasma was nearly equal to that in brain and their brain/plasma ratio was time-independent, linear relationships between the brain concentrations and the unbound plasma concentrations for each drug could be obtained (Fig. 4). These results suggest that these xanthine derivatives have no saturable brain uptake. The penetration ratio, which is calculated as the slope of the regression line in Fig. 4, was 0·557 (r = 0·794) for theophylline, 0·071 (r = 0·643) for enprofylline, 0·227 (r = 0·943), 0·176 (r = 0·711) for MPX and 0·236 (r = 0·968) for oxpentifylline.

The biological activities of xanthine derivatives are summarized in Table 4. The potency of cAMP-PDE inhibitory activities for theophylline, enprofylline and oxpentifylline was approximately one-half that of MPX. Enprofylline and oxpentifylline exhibited a low affinity for adenosine A_1 receptors in contrast with theophylline and MPX. MPX showed the strongest activity with regard to both biological activities in the xanthines studied.

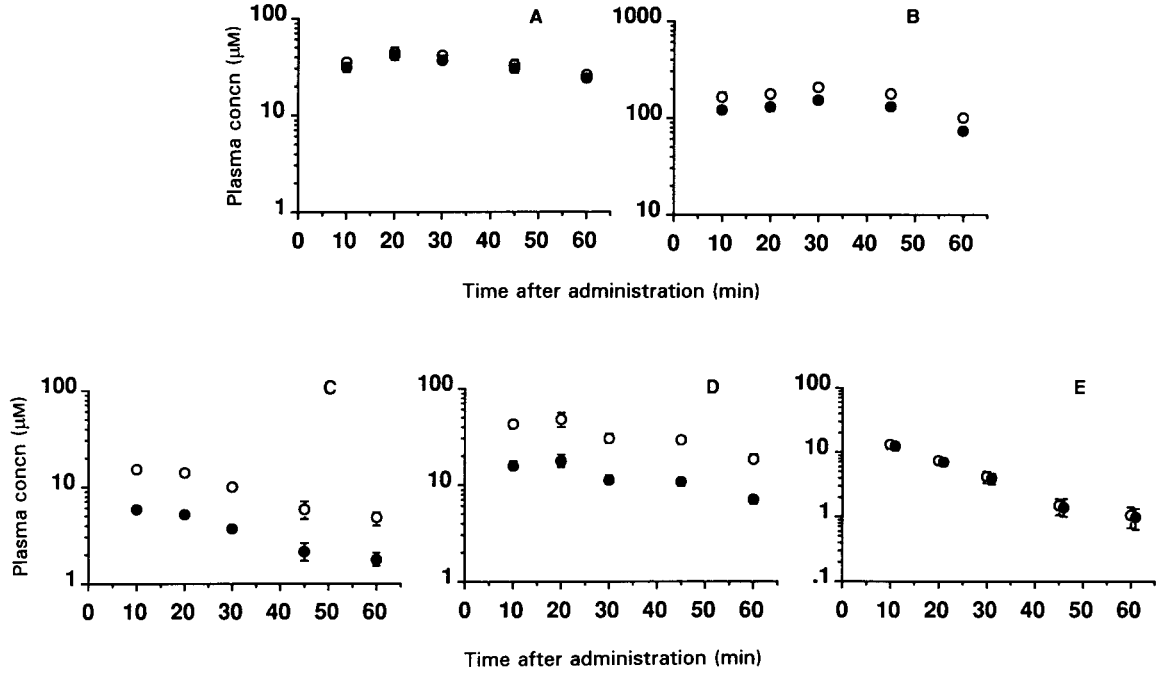

FIG. 2. Mean semilogarithmic plots of plasma concentration–time data for total (O) and unbound (●) concentrations of xanthines after a single oral administration. Each point represents mean ± s.e.m. for five determinations. A. Theophylline (10 mg kg^{-1}); B. enprofylline (30 mg kg^{-1}); C. MPX (3 mg kg^{-1}); D. MPX (10 mg kg^{-1}); E. oxpentifylline (100 mg kg^{-1}).

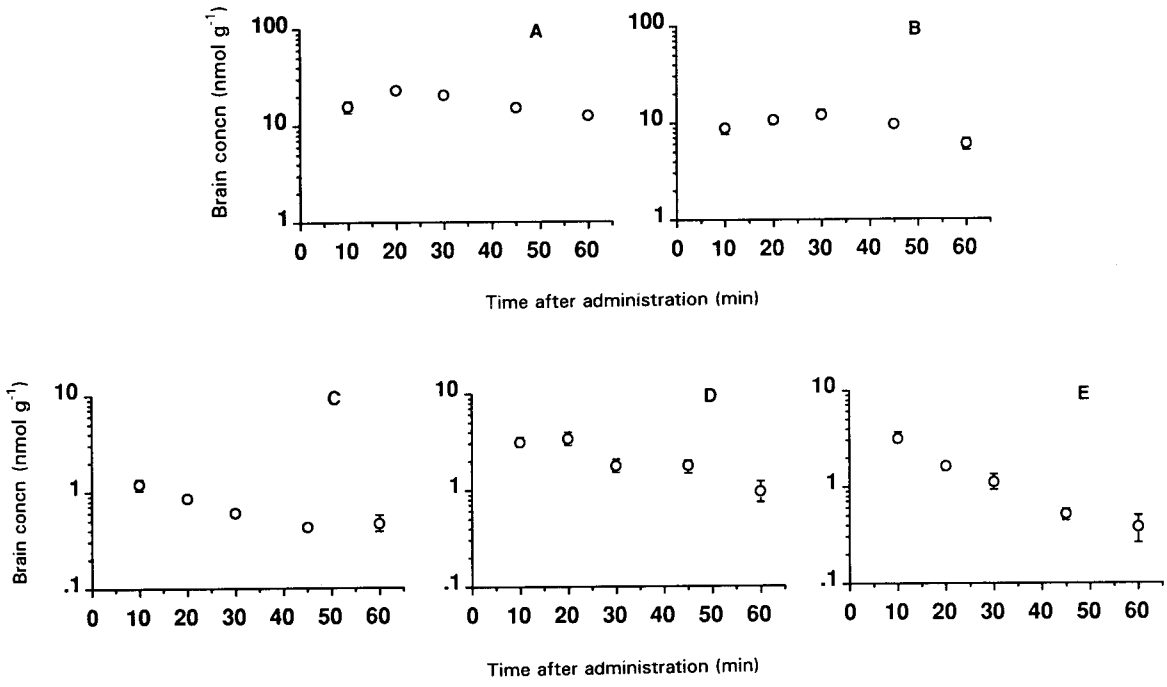

FIG. 3. Mean semilogarithmic plots of brain concentration–time data of xanthines after a single oral administration. Each point represents the mean ± s.e.m. for five determinations. A. Theophylline (10 mg kg^{-1}); B. enprofylline (30 mg kg^{-1}); C. MPX (3 mg kg^{-1}); D. MPX (10 mg kg^{-1}); E. oxpentifylline (100 mg kg^{-1}).

Discussion

The purpose of the present study was to characterize the brain distribution characteristics and motor activity in mice of the four selected xanthine derivatives, as related to their pharmacological actions in the central nervous system (CNS).

The permeability through biological membranes of a drug is generally considered to depend on such factors as protein-binding, hydrophobicity and the molecular size of the drug. The present study demonstrated that these xanthine derivatives showed relatively low protein-binding potency, which was also concentration-independent, in mice. This differs

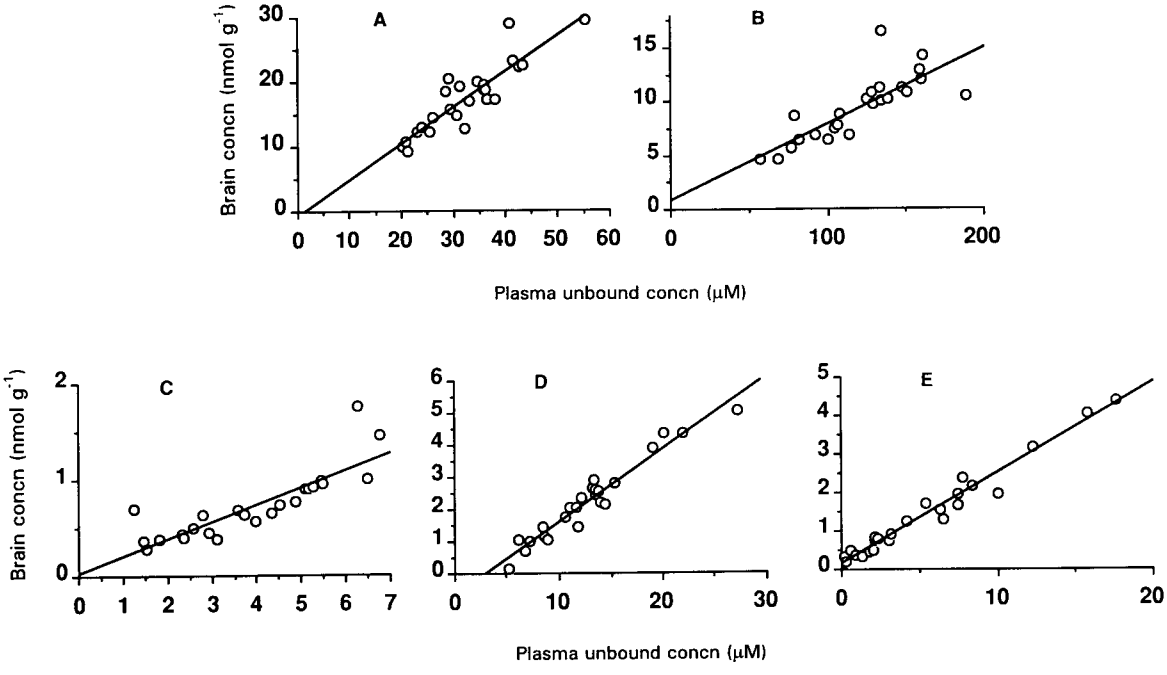

FIG. 4. Relationship between brain and unbound plasma concentrations for xanthines after a single oral administration. A. Theophylline (10 mg kg^{-1}); B. enprofylline (30 mg kg^{-1}); C. MPX (3 mg kg^{-1}); D. MPX (10 mg kg^{-1}); E. oxpentifylline (100 mg kg^{-1}).

Table 4. Pharmacological activities of xanthines.

Xanthine	cAMP-PDE inhibitory activity[a] K_i (μM)	Affinity for adenosine receptor[b] K_i (μM)
Theophylline	134·2 ± 10·4	44·6 ± 7·5
Enprofylline	112·4 ± 3·5	> 1000
MPX	48·7 ± 3·5	26·4 ± 7·8
Oxpentifylline	111·4 ± 10·6	> 1000

Each value represents mean ± s.e.m. of four measurements. [a] The inhibitory activity was exhibited against the high affinity form with low K_m of 1·17 μM and low V_{max} of 303·0 pmol min^{-1} (mg protein)$^{-1}$. [b] The Scatchard plot of the CPX binding to the brain membrane preparation was linear with a K_d value of 3·97 nM and a B_{max} value of 290·1 fmol (mg protein)$^{-1}$.

from the reported data for enprofylline and MPX in rats and guinea-pigs (Hasegawa et al 1991a; Nadai et al 1991), but may be explained by species differences. This study confirmed previous reports (Hasegawa et al 1990a, 1991b) that there is a close relationship between the protein-binding potency and hydrophobicity of these xanthine derivatives.

We evaluated the brain distribution characteristics of these xanthine derivatives by measuring both the unbound plasma concentrations (recalculated from their total plasma concentrations) and the mean unbound fractions, since marked differences in unbound fractions were observed among these xanthines. The plasma and brain concentration–time profiles of each xanthine derivative were essentially the same while a linear relationship observed between the unbound plasma and brain concentrations indicated that their brain distribution depends on plasma concentration. Theophylline had the highest brain penetration ratio (0·557) whereas enprofylline had the lowest ratio (0·071). These results are close to those in man (Auritt et al 1985). The plasma concentration range of theophylline in this experiment is consistent with that in brain extracellular fluids (58 and 63 μM, respectively) reported by Snyder et al (1981) and Stahle et al (1990). It has also been reported that a dose of 3 mg kg^{-1} theophylline attained a brain extracellular concentration range of 8–14 μM (Stahle et al 1990). From these data, the range of theophylline concentration in brain extracellular fluids obtained from this study using a dose of 10 mg kg^{-1} can be predicted to be 26·4–46·2 μM, indicating that the plasma concentration of theophylline reflects its extracellular concentration in the brain. In addition, assuming that the brain-barrier permeability for theophylline with a brain penetration ratio of 0·557 is complete (100%), the brain-barrier permeability for the other three xanthine derivatives are calculated as 12·8% for enprofylline. 40·8 and 31·6% for the two doses of MPX, and 42·4% for oxpentifylline.

Ionization is an important factor which influences blood-brain-barrier penetration by making the drug hydrophilic thus favouring its retention in the water phase. Moreover, ionization can alter the affinity of the drugs for the plasma-protein-binding sites. As it is generally understood that there is a direct relationship between the diffusion into CNS and the hydrophobicity of drugs, the hydrophobic properties of these xanthine derivatives were determined. When we consider the results showing that MPX has the highest hydrophobicity, we can expect it to be the most diffusible drug in the brain. However, we have failed to explain why MPX brain penetration was lower than that of theophylline in this stage. Enprofylline has the highest hydrophobicity and protein-binding potency after MPX, but showed the lowest brain penetration (brain/plasma ratio = 0·071). This result may be explained by efflux of enprofylline from brain to blood by means of an active transport system not applicable to the other xanthines. Enprofylline is a non-metabolized drug, mainly excreted in an unchanged form in the urine by an active tubular secretion mechanism in man and in animals (Borga et al 1986; Nadai et al 1991; Wang et al 1993). The relevant examples may be penicillin and azidothymidine which are also eliminated from the body by an active tubular-secretion system. Indeed, it has also been indicated that an active efflux system readily transfers these compounds from brain into blood (Spector & Lorenzo 1974a, b; Hedaya & Sawchuk 1989; Wong et al 1993). In fact, Laursen et al (1989) have suggested this possibility, showing that the CSF concentration of enprofylline was much lower than that of theophylline in man. Other than this, the degree of brain penetration of oxpentifylline, which exhibits the lowest hydrophobicity and protein-binding potency, was lower than that of theophylline. Accordingly, the hydrophobicity of the xanthine derivatives tested may play some role, though not a major one, in their brain distribution, since the hydrophobicity did not correlate with the brain-penetration ratio.

Theophylline, known to be a potent CNS-stimulant drug, induced an increase in motor activity following a single oral administration at a dose of 10 mg kg^{-1}. It can be considered that theophylline diffuses well into the brain and can compete with adenosine A_1 receptors because a sufficient concentration in brain for antagonism was attained (Fredholm & Persson 1982), while the brain concentration is not enough to exhibit cAMP-PDE inhibitory activity (> 100 μM). These observations are further evidence for the hypothesis that it is unlikely that the inhibition of cAMP-PDE accounts for the motor activity of theophylline, the blocking effect of adenosine receptors being the likelier explanation. In the present study enprofylline showed a low affinity for adenosine A_1 receptors in mouse brain membranes, as it had in those of guinea-pigs (Miyamoto et al 1993), while its concentration in the brain was less than the concentration required to antagonize the adenosine A_1 receptors (K_i > 1000 μM). Such findings suggest that enprofylline fails to display any changes in motor activity. Similar findings have been reported for doxofylline, a xanthine derivative which has also shown a low affinity for adenosine A_1 receptors and also could not alter motor activity in rats (Cravanzola et al 1989).

Oxpentifylline had a lower cAMP-PDE inhibitory activity (K_i > 100 μM) and affinity for adenosine A_1 receptors (K_i > 1000 μM), results in agreement with those obtained by using the brain membranes prepared from guinea-pigs as reported previously (Miyamoto et al 1993). In this study, the observed maximum brain concentration of oxpentifylline after oral administration at a dose of 100 mg kg^{-1} was only approximately 3 nM g^{-1}, which, although its brain penetration ratio was relatively high (0·236), was lower than the

concentration for antagonizing adenosine A_1 receptors. These observations, indicating that oxpentifylline could not induce any significant changes in motor activity in mice, are not surprising because that drug is not a bronchodilator. It seems to be reasonable to conclude that its action is not mediated by adenosine. In the clinical use of oxpentifylline, its maintenance dose as a vasodilator is 1–3 mg kg^{-1}, which is low compared with the dose used in this study. It is likely that oxpentifylline does not induce side-effects mediated by adenosine. Hand et al (1989) have reported that oxpentifylline does not bind to nucleoside transport receptors in the membrane. Unexpectedly, an administration of 10 mg kg^{-1} MPX, which had the highest affinity for adenosine A_1 receptors, decreased motor activity. There are experimental data indicating that 1-methyl-3-isobutylxanthine (IBMX), a potent PDE IV inhibitor and a potent adenosine receptor antagonist, exhibits a depressant effect and low brain penetration (Beavo et al 1970; Snyder et al 1981; Choi et al 1988) and that rolipram, a nonxanthine and a highly selective inhibitor for PDE IV, produces behavioural depression (Choi et al 1988). These experimental data and the observed behaviour for MPX in this study may suggest that the inhibition of PDE IV activity is one of the factors effective in decreasing motor activity. However, it has also been reported that an adenosine A_2-receptor agonist induced behavioural depression and the behavioural depression was inhibited by theophylline (Ferre et al 1991). These results suggest the possibility that adenosine A_2 receptors are related to the locomotor effects of xanthine, although their affinities for adenosine A_2 receptors were not measured in this study. Further investigation into the precise mechanisms for the locomotor effects of xanthines will be needed.

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