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PHOSPHODIESTERASE INHIBITORY PROFILE OF SOME RELATED XANTHINE DERIVATIVES PHARMACOLOGICALLY ACTIVE ON THE PERIPHERAL MICROCIRCULATION

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Abstract—The cyclic nucleotide phosphodiesterase (PDE) inhibitory profile of four related xanthine derivatives: pentoxifylline (BL 191), propentofylline (HWA 285), torbafylline (HWA 448) and albifylline (HWA 138), pharmacologically active on the peripheral and/or cerebral microcirculation was established using the four main PDE isoforms present in rat heart cytosol. HPLC on a Mono Q ion-exchange column resolved four separate cyclic nucleotide PDE activities: a calmodulin-activated fraction (PDE I), a cGMP-stimulated fraction (PDE II), a cAMP-specific rolipram-sensitive fraction (PDE IV) and a cGMP-inhibited fraction (PDE III). Among the four compounds studied, only torbafylline and pentoxifylline inhibited more efficiently the calcium plus calmodulin-stimulated than the basal activity of PDE I. The four xanthine derivatives inhibited more potently the cGMP-stimulated than the basal activity of the cGMP-stimulatable PDE II, propentofylline being the most inhibitory (IC_{50}: 20 μM). Except for propentofylline, which exhibited a marked selectivity toward the rolipram-sensitive PDE IV versus the cGMP-inhibited PDE III, the other xanthines modestly (IC_{50} in the 10^{-4} M range) inhibited both cAMP-specific isoforms with similar potency. Propentofylline proved to be the best inhibitor whatever the considered isoform whereas torbafylline exhibited the weakest inhibitory potency with, however, some selectivity for PDE I.

Key words: xanthine related compounds; pentoxifylline; propentofylline; torbafylline; albifylline; phosphodiesterase isoenzyme inhibition

Among alkylated xanthines known to exhibit a great variety of pharmacological properties, four structurally related compounds: pentoxifylline (BL 191), propentofylline (HWA 285), torbafylline (HWA 448) and albifylline (HWA 138) have been reported to show protective effects in various types of vascular disturbances. Whereas pentoxifylline has beneficial effects both in peripheral and in cerebral vascular diseases [1, 2], propentofylline exhibits marked neuroprotective effects [3], and torbafylline proves to be more specific on the peripheral microcirculation [4, 5]. Both pentoxifylline and albifylline have beneficial effects on the cardiocirculatory dysfunctions associated with septic shock [6]. At the biochemical level, most of the alkylxanthines either inhibit cyclic nucleotide PDE§ and/or act as antagonists for adenosine receptors [7]. Prototypical xanthines such as theophylline and IBMX are non-selective inhibitors of the various PDE isoforms [8]. However, some 7- and 8-substituted xanthines have been shown to be relatively selective for the calmodulin-stimulated (type I) PDE [9, 10]. Other xanthines such as 1-, 3-, 7-tripropylxanthine have been reported as selective inhibitors of the calcium and calmodulin-independent PDE isoforms [7]. Among xanthine derivatives which have been developed in recent years, denbufylline [1,3-dibutyl-7-(2-oxopropyl) xanthine] has been shown to be highly selective for the cAMP specific rolipram-inhibitable type IV PDE isoform from vascular smooth muscle and brain [11, 12], with relatively low affinity for A_1 and A_2 adenosine receptors [12]. In contrast, xanthines with 8-phenyl and 8-cycloalkyl substituents proved to be far more effective (about 10,000-fold) as adenosine receptor blocking agents (K_i in the nanomolar range) than as PDE inhibitors [13]. However two of them, 1,3-diethyl-8-phenylxanthine and 1,3-dipropyl-8-cyclopentyl xanthine exhibited a marked selectivity for PDE IV as compared with the other PDE isoenzymes with IC_{50} values near that of rolipram [13]. Several reports have examined the ability of pentoxifylline and/or propentofylline to inhibit cAMP-PDE activity in various tissues including human umbilical cord vessels [14], guinea pig trachealis muscle [15], brain, heart, aorta and platelets from rat [16]. However,

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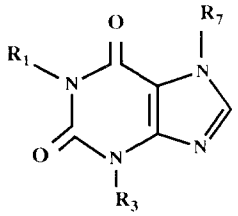
§ Abbreviations: IBMX, 3-isobutyl-1-methylxanthine; PDE, cyclic nucleotide phosphodiesterase; CAM-PDE, phosphodiesterase activity stimulated by calmodulin plus calcium; CGS-PDE, phosphodiesterase activity stimulated by cGMP; ROI-PDE, phosphodiesterase activity selectively inhibited by rolipram; CGI-PDE, phosphodiesterase activity selectively inhibited by cGMP; DTT, dithiothreitol; PMSF, phenyl methyl sulphonyl fluoride; TNF-α, tumour necrosis factor-α; LPS, lipopolysaccharide.

Table 1. The chemical structure of the xanthines used in this study



Generic name		R_1	R_3	R_7
HWA 285	Propentofylline	CH_3-CO-$(CH_2)_4$-	CH_3	CH_3-CH_2-CH_2-
HWA 448	Torbafylline	$(CH_3)_2$-COH-$(CH_2)_4$-	CH_3	CH_3-CH_2-O-CH_2-
HWA 138	Albifylline	$(CH_3)_2$-COH-$(CH_2)_4$-	CH_3	H-
BL 191	Pentoxifylline	CH_3-CO-$(CH_2)_4$-	CH_3	CH_3-

none of these studies addressed the question of an eventual selectivity of inhibition toward the different PDE isoenzymes. In a recent report, Cortijo *et al.* [17] have compared the inhibitory potency of pentoxifylline and theophylline to that of rolipram and denbufylline toward PDE I, II and IV isolated from human bronchus. As expected, only rolipram and denbufylline proved to be selective inhibitors of PDE IV, pentoxifylline exhibiting IC_{50} values in the 50–100 μM range, whatever the considered PDE isoform. Only recently, Semmler *et al.* [18] have shown that torbafylline and albifylline were also able to inhibit a commercially available cAMP-PDE from beef heart with potencies similar to that of pentoxifylline and theophylline, but the question of an eventual selectivity was not envisaged.

Thus, the present work was undertaken in order to compare the inhibitory potencies of the four related xanthines (BL 191, HWA 285, HWA 448 and HWA 138) toward the various PDE isoforms. Rat heart is a convenient material for this purpose since it contains four of the five main families of PDE isoforms. HPLC consistently resolves rat heart cytosol into four well-characterized PDE isoforms: a calcium plus calmodulin-dependent type I isoform CAM-PDE or PDE I, a cGMP-stimulated isoform CGS-PDE or PDE II, a high affinity cAMP-specific isoform sensitive to rolipram inhibition (ROI-PDE or PDE IV) and a high affinity cAMP-specific isoform sensitive to cGMP inhibition (CGI-PDE or PDE III) [19]. Therefore, in the present study we have compared the inhibitory potency of the four related xanthine derivatives toward the various PDE isoforms from rat heart supernatant separated by HPLC using a Mono-Q ion-exchange column.

MATERIALS AND METHODS

Materials. [8-^{3}H]cAMP (28 Ci/mmol), [8-^{3}H]-cGMP (15 Ci/mmol) and [U^{14}C]adenosine (571 mCi/mmol) were obtained from Amersham (Les Ullis, France). [U^{14}C]Guanosine (528 mCi/mmol) was from Du Pont de Nemours (Paris, France). Unlabelled cyclic nucleotides, 5′-nucleotidase (*Ophiophagus hannah* venom) were from the Sigma Chemical Co. (La Verpillère, France). Pentoxifylline: 3,7-dimethyl-1-(5-oxohexyl) xanthine (BL 191), propentofylline: 3-methyl-1-(5-oxohexyl)-7-propylxanthine (HWA 285), torbafylline: 3-methyl-1-(5-hydroxy - 5 - methylhexyl) - 7 - ethoxymethyl xanthine (HWA 448) and albifylline: 3-methyl-1-(5-hydroxy-5-methylhexyl) xanthine (HWA 138) were gifts from Hoechst AG (Wiesbaden, Germany). The chemical structure of the xanthines used in this study are shown in Table 1.

Tissue preparations. Male Sprague–Dawley rats (250–300 g) were decapitated. Hearts were perfused with 0.15 M NaCl through the aorta to remove the blood. The ventricles were minced in 5 vol. of 10 mM Tris–HCl buffer containing 0.32 M sucrose, 1 mM EDTA, 5 mM DTT and 0.1 mM PMSF at pH 7.5. The suspension was homogenized in a glass–glass Potter–Elvejhem. The homogenate was then centrifuged at 105,000 *g* for 60 min. The 105,000 *g* supernatant was stored at −75° until injection on the HPLC column.

Isolation of PDEs. The cytosolic fraction from rat ventricles (5–8 mg of proteins) was loaded at 1 mL/min on a Mono Q HPLC column which had been previously equilibrated with buffer A (50 mM Tris–HCl, 2 mM EDTA, 14 mM 2-mercaptoethanol, 0.1 mM PMSF, pH 7.5). Under these conditions, greater than 95% of the PDE activity was bound to the column. PDE activity was eluted at a flow rate of 1 mL/min using the following step by step and linear gradients of NaCl in buffer A: 25 mL of 0.16 M NaCl, 20 mL of 0.23 M NaCl, 30 mL from 0.23 to 0.29 M NaCl, 15 mL of 0.29 M NaCl, 30 mL from 0.29 to 0.50 M NaCl. The separation was done at 4°. Fractions of 1 mL were collected and stored at −75° in the presence of 20% glycerol. The fractions were tested for PDE activity, and the peaks containing the different isoenzymes were identified. Fractions containing preferentially one isoenzyme were pooled.

PDE assay. PDE activity was assayed by a two-step radioisotopic procedure modified by Prigent *et al.* [20]. cAMP-PDE and cGMP-PDE activities were measured with a substrate concentration of 0.25 μM. To evaluate the cGMP-stimulated PDE activity, assays were performed with 5 μM cAMP in the absence or presence of 5 μM cGMP. Xanthine derivatives were dissolved in DMSO. The stock solutions were appropriately diluted with 40 mM Tris–HCl buffer so that the final DMSO concentration in the PDE assay did not exceed 1%. At this concentration, DMSO had no significant effect on the PDE activity of any of the fractions. The inhibitory potency of the four xanthine derivatives was examined on each separated isoform. The IC_{50} values (concentration of a drug which inhibited 50% of the enzymatic activity) were calculated by plotting the percentage of residual enzymatic activity versus the logarithmic concentration of the drug. Confidence limits (95%) for the IC_{50} values were determined by linear regression analysis according to Hubert [21].

RESULTS

As previously reported by us and others, HPLC fractionation routinely resolves four peaks of PDE activity from rat heart cytosol. The HPLC profiles shown in Fig. 1A and B are similar to those previously described by Nicholson *et al.* [22], Bode *et al.* [23] and by us [19]. The first peak eluting with 0.16 M NaCl preferentially hydrolysed cGMP when substrates were added at a low concentration (0.25 μM). The cyclic nucleotide hydrolysing activity of this peak was stimulated about 2-fold by 100 U calmodulin in the presence of 5×10^{-4} M $CaCl_2$ with respect to the basal level measured in the presence of 10^{-3} M EGTA (not shown). Pooled fractions from this peak (15–33) were used as a source of calmodulin-stimulated PDE. The second peak eluted with 0.23 M NaCl hydrolysed both cAMP and cGMP with similar rates when substrates were present at 0.25 μM. As shown in Fig. 1B, cAMP hydrolysis by this peak was substantially increased (5-fold) by 5 μM cGMP. Thus, fractions 40–55 were pooled and used as a source of cGMP-stimulated PDE activity. Both the third peak eluted with 0.29 M NaCl and the fourth peak eluted by the linear portion of the gradient between 0.29 and 0.50 M NaCl specifically hydrolysed cAMP. The cAMP-PDE activity of peak 3 (0.29 M NaCl) was sensitive to rolipram inhibition (IC_{50} = 6 μM) and was weakly inhibited by cGMP. Conversely, the cAMP-PDE activity of peak 4 was markedly inhibited by cGMP ($IC_{50} = 0.3$ μM) and was far less sensitive to rolipram inhibition ($IC_{50} > 200$ μM). Thus, fractions 81–96 and fractions 124–136 were pooled and used as a source of rolipram-inhibitable PDE and cGMP-inhibitable PDE, respectively.

Among the four xanthine derivatives tested, compound HWA 138 exhibited the weakest inhibitory potency toward the calmodulin-dependent PDE isoform (Table 2). IC_{50} values obtained in the presence of EGTA (basal activity) or in the presence of calcium plus calmodulin (stimulated activity) were similar, thus indicating that this compound had no anti-calcium properties. Compound HWA 285 was the most effective with similar IC_{50} values (about 30 μM) either in the presence of EGTA or in the presence of calmodulin. In contrast, both BL 191 and HWA 448 inhibited more efficiently the calmodulin-stimulated PDE activity than the basal activity (IC_{50}: 164 vs 331 μM and 56 vs 93 for BL 191 and HWA 448, respectively).

The basal activity of the cGMP-stimulated PDE measured in the absence of the allosteric activator cGMP, appeared rather insensitive to the xanthine derivatives tested (Table 2). Among them, compound HWA 285 was the most efficient with an IC_{50} of 220 μM. However, in the presence of cGMP, the inhibitory potency of the four compounds was markedly increased. IC_{50} values being comprised in the 20–150 μM range. Especially, compounds HWA 285 and HWA 138 were about 10-fold more potent inhibitors of cAMP hydrolysis in the presence than in the absence of cGMP (IC_{50} A vs IC_{50} A + G: 11 and >10 for HWA 285 and HWA 138, respectively). These results indicate that these xanthine derivatives have a greater affinity for the allosteric cGMP binding site than for the catalytic sites hydrolysing both cGMP and cAMP. This pecularity seems to be a characteristic of most alkylxanthines [24].

Among the four xanthine derivatives tested, only compound HWA 285 inhibited more potently the rolipram-sensitive than the cGMP-inhibited PDE isoform (Table 2) (ROI-PDE: $IC_{50} = 20$ μM vs CGI-PDE: $IC_{50} = 100$ μM). The three other compounds did not exhibit any selectivity toward one or the other cAMP-specific PDE isoforms, compound HWA 448 being the less potent inhibitor of both isoforms. The selectivity of compound HWA 285 was even higher when assays on ROI-PDE were performed in the presence of 100 μM cGMP to limit cross-contamination by CGI-PDE and assays on CGI-PDE were done in the presence of 100 μM rolipram to limit cross-contamination by ROI-PDE. In these assay conditions, HWA 285 exhibited a slightly lower IC_{50} value for ROI-PDE: 8.2 μM (95% confidence limits 6.7–10.1) and a markedly higher IC_{50} value for CGI-PDE: 300 μM (95% confidence limits 210–340). In contrast, the IC_{50} values of the three other compounds for ROI-PDE and CGI-PDE were not significantly modified as expected for non-selective inhibitory compounds.

DISCUSSION

None of the xanthine derivatives tested in the present study proved to be a potent inhibitor of the various PDE isoforms present in rat heart cytosol, all the measured IC_{50} values being higher than 1 μM. Our results are in good agreement with those reported by others for different series of substituted xanthines. Thus, a series of twenty-one 1,3,7,8-substituted xanthines exhibited IC_{50} values ranging from 4 to 180 μM toward rat brain PDE [25]. Choi *et al.* [7] have reported IC_{50} values of several 1,3,7-substituted xanthines ranging from 5 to 750 μM for the rat brain calcium-dependent PDE isoform and from 2 to 670 μM for the rat brain calcium-independent PDE activity. Using crude PDE extracts from guinea pig trachealis muscle, Miyamoto *et al.* [15] have shown that various 3-, 7-substituted 1-(5′-

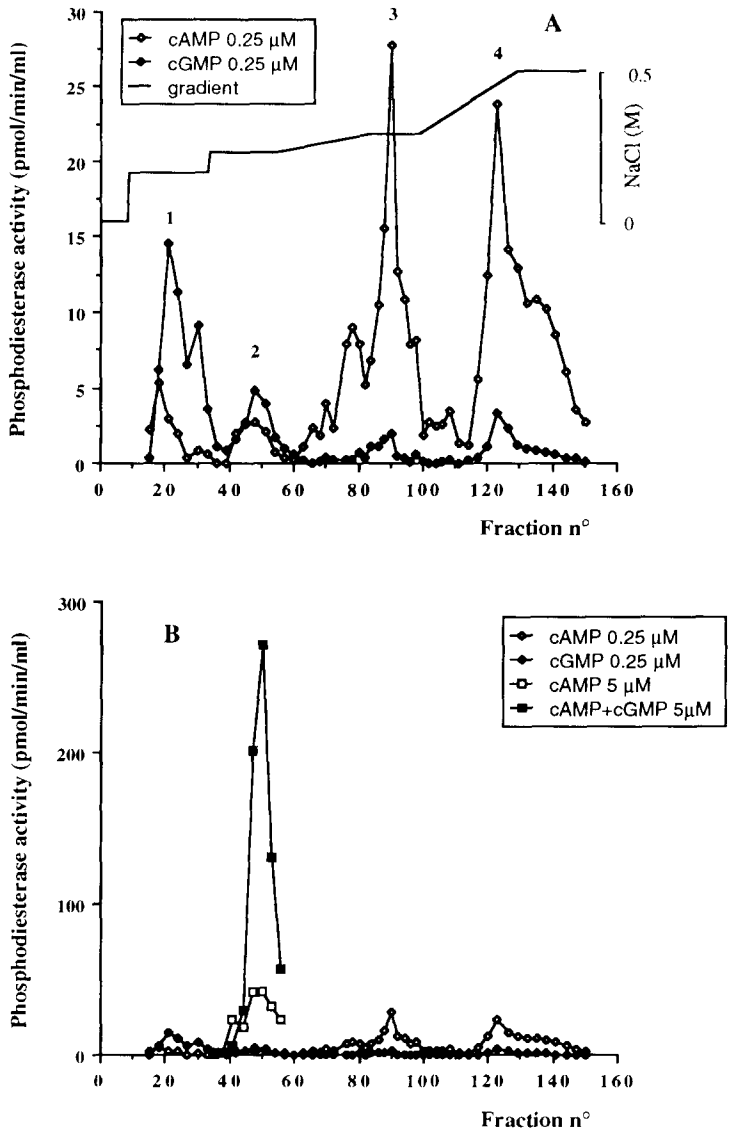


Fig. 1. Chromatographic resolution of PDE activity from the soluble fraction of rat cardiac ventricle eluted from Mono Q ion-exchange column by HPLC. (A) PDE activity was assayed with 0.25 μM cAMP (open diamonds) or 0.25 μM cGMP (solid diamonds). (B) cGMP-stimulated activity. PDE activity of fractions 35–56 was assayed with 5 μM cAMP as substrate without (open squares) or with 5 μM cGMP (solid squares).

oxohexyl)xanthines exhibited K_i values ranging from 10 to 100 μM. However, none of these studies were performed with chromatographically separated and semi-purified PDE isoforms. Pentoxifylline (BL 191) and propentofylline (HWA 285) are two closely related xanthine derivatives differing only by the nature of the substituent present at position 7 of the xanthine nucleus: a methyl group for the former and a propyl group for the latter. Whereas pentoxifylline proved to be only a modest inhibitor of the various PDE isoforms without any marked selectivity, propentofylline proved to be almost as efficient as rolipram in inhibiting the rolipram-sensitive cAMP-specific PDE isoform. However, in contrast with rolipram which did not significantly inhibit (IC_{50} = 493 μM) the calcium and calmodulin-dependent PDE isoform [26], propentofylline also markedly inhibited this PDE isoform, either in the presence or absence of calcium plus calmodulin. The relatively strong affinity of HWA 285 for the cAMP-specific rolipram-sensitive PDE isoform does not fit very well with the working model previously proposed by Wells *et al.* [10], predicting that xanthines with 7- or 8-substituents should exhibit reduced affinity for cAMP-specific PDE isoforms. It is noteworthy that denbufylline, a selective inhibitor of the rolipram-sensitive PDE, bears a 2-oxopropyl group at position 7 of the xanthine nucleus. Similar exceptions to the

Table 2. Effects of the xanthine derivatives on the activity of the separated PDE isoforms from rat cardiac ventricle

	CAM-PDE (PDE I) IC_{50} (μM)			CGS-PDE (PDE III) IC_{50} (μM)			cAMP-specific PDE IC_{50} (μM)		
Compounds	cGMP 0.25 μM + EGTA*	cGMP 0.25 μM + CAM + Ca^{2+}†	IC_{50} EGTA/ IC_{50} CAM	cAMP 5 μM	cAMP 5 μM + cGMP 5 μM	IC_{50} cA/IC_{50} cA + cG	ROI-PDE (PDE IV) cAMP 0.25 μM	CGI-PDE (PDE III) cAMP 0.25 μM	IC_{50} ROI/ IC_{50} CGI
HWA 285	29.3 [25.8–33.3]	32.1 [23.9–43.1]	0.91	220.8 [179.6–271.5]	19.2 [10.9–33.8]	11.5	20.9 [17.2–25.2]	97.0 [80.4–117.2]	0.22
HWA 448	92.7 [72.7–118.2]	55.6 [43.9–70.5]	1.67	610.9 [479.7–778.0]	153.0 [123.8–189.4]	4.0	177.3 [153.0–210.2]	294.4 [264.9–327.3]	0.60
HWA 138	380.5 [292.7–494.8]	349.9 [305.1–401.3]	1.09	>800	81.9 [68.9–97.2]	>10	138.4 [123.0–155.6]	137.1 [126.6–148.5]	1.00
BL 191	333.1 [189.2–579.4]	164.2 [129.6–208.3]	2.02	347.5 [211.8–570.2]	49.8 [41.0–60.4]	7.0	111.7 [89.9–138.8]	157.0 [140.8–175.2]	0.71
Rolipram		493‡		280§			6.0 [4.7–7.8]	>200	
cGMP	—	—		—	—		>100	0.3 [0.02–3.0]	

IC_{50} values (concentration that inhibits substrate hydrolysis by 50%) were determined from concentration–response curves. Enzyme activity was measured as described in the Materials and Methods. Results are representative of three determinations obtained for two different enzymatic preparations. Values between brackets represent 95% confidence limits.

* EGTA concentration was 1 mM; † calmodulin was used at 100 U/assay in the presence of 500 μM $CaCl_2$; ‡ from Ref. 26; § from Ref 26 (assays were done with 0.25 μM cGMP as substrate).

model proposed by Wells *et al.* [10] were pointed out by Ukena *et al.* [13] who have demonstrated that some xanthines substituted with a phenyl or a cyclopentyl group at position 8 exhibited a marked selectivity for the rolipram-sensitive PDE compared to the other PDE isoenzymes, with an efficacy similar to that of rolipram (IC_{50} in the 10 μM range). Torbafylline (HWA 448) and albifylline (HWA 138) also present closely related structures: the same substituent at positions 1 and 3 of the xanthine nucleus, and an ethoxymethyl group vs hydrogen at position 7 for compounds HWA 448 and HWA 138, respectively. These two xanthine derivatives were weak inhibitors of the different PDE isoforms. However, the ethoxymethyl group at 7 confers to the HWA 448 molecule some affinity for the calmodulin-dependent PDE, while compound HWA 138 does not exhibit any selectivity. In addition, compound HWA 448 also exhibited some anti-calcium properties since it inhibited more potently the calcium plus calmodulin-stimulated than the basal activity of this PDE isoform. Thus, the presence of a substituent with more than one carbon atom at postion 7 of the xanthine nucleus seems to confer some preference for the calmodulin-dependent PDE. Both, propentofylline (R_7 = propyl) and torbafylline (R_7 = ethoxymethyl) inhibit the calmodulin-dependent PDE with IC_{50} values lower than 100 μM whereas pentoxifylline (R_7 = methyl) and albifylline (R_7 = H) are only weak inhibitors of this isoform.

Since most xanthine derivatives are only weak and non-selective as PDE inhibitors, it is generally thought that their pharmacological activities are primarily due to blockade of adenosine receptors or inhibition of adenosine re-uptake [7]. In several circumstances however, the PDE inhibiting properties of xanthines have clearly been shown to be a part of their mechanism of action. Out of the four xanthine derivatives tested in the present study, propentofylline proved to be the best inhibitor whatever the considered PDE isoform and the only one able to exhibit a relative affinity for the rolipram-sensitive PDE as compared with the cGMP-inhibited isoform. Thus, it might be of interest to compare the pharmacological profile of propentofylline to that of selective inhibitors of the rolipram-sensitive PDE isoenzyme. Rolipram was initially described by Schwabe *et al.* [27] as a potent cAMP-PDE inhibitor in homogenates and tissue slices from rat brain, able to increase basal level of cAMP and to potentiate cAMP accumulation induced by norepinephrine, isoproterenol, histamine and adenosine, without significant effect on the release of adenosine. Rolipram is now well known for its antidepressant properties both in animals [28, 29] and in humans [30]. Rolipram behavioural syndrome in rats is characterized by locomotor depression, hypothermia and increase maintenance activity (forepaw-shaking, grooming, head twitches) [28, 29]. This peculiar behavioural syndrome is thought to reflect an enhanced availability of cerebral cAMP [28]. Interestingly, the non-selective PDE inhibitors theophylline and IBMX which induced hyperactivity in rats at low dosages due to their adenosine antagonist action, also induced head twitches, forepaw-shaking and grooming at higher dosages, indicating the prevalence of the cAMP inhibiting action at high dosages [28]. In addition, inhibitors of the rolipram-sensitive PDE are known to improve the performance of animals in certain paradigm of cognition through a cAMP-mediated enhancement of responses to glutamate [22]. Besides its protective effects against neuronal damage induced by cerebral ischemia, propentofylline has also been shown to improve learning and memory in aged animals and in animals with genetic learning impairment [31]. It is noteworthy that propentofylline is currently being evaluated for the treatment of senile dementia, this potential being also under investigation for the ROI-PDE specific inhibitor, denbufylline [22]. Besides their potential usefulness in the treatment of central disorders such as depression and dementia, selective inhibitors of the rolipram sensitive PDE seem of peculiar interest as anti-inflammatory agents. Indeed, rolipram has recently been described as a remarkably potent suppressor of LPS-induced synthesis of TNF-α by human mononuclear cells *in vitro*, with IC_{50} value in the 130 nM range [32]. This inhibitory effect toward TNF-α production is able to explain all the protective effects of rolipram observed in an experimental model of Acute Respiratory Distress Syndrome (ARDS), namely reduction of LPS-induced pulmonary edema, alveolar hemorrhage and mortality [33]. Pentoxifylline [34] as well as propentofylline [35] have been shown to block the secretion of TNF-α in LPS-stimulated peripheral blood mononuclear cells and monocytes. Since excessive microglia stimulation seems to be involved in the pathophysiology of degenerative dementia, the *in vivo* modulation of TNF-α level might be physiologically relevant. Recently, a high degree of correlation for pentoxifylline, albafylline and torbafylline has been pointed out between their potency to suppress TNF-α production in cell culture system and their potency to inhibit PDE activity, arguing for a crucial role of PDE inhibition in the suppression of TNF-α synthesis [18]. The protective effect of propentofylline against neuronal damage induced by cerebral ischemia has been previously attributed to the inhibition of adenosine uptake [36]. Besides, propentofylline has been reported to have approximately 10-fold lower affinity for A_2 receptors (K_i = 200 μM) than for A_1 receptors (K_i = 20 μM) [37]. It can be assumed that in therapeutic doses propentofylline enhances extracellular adenosine level and antagonizes inhibitory adenosine A_1 receptors while leaving stimulatory adenosine A_2 receptor only marginally affected, thus reinforcing the blood flow promoting properties of endogenous adenosine. However, the peculiar PDE inhibitory profile of propentofylline seems to be another important component of its pharmacological mode of action. Besides its marked selectivity for the rolipram-sensitive PDE isoform, propentofylline also efficiently inhibited the calmodulin-dependent PDE and the cGMP-stimulated PDE in the presence of cGMP with an IC_{50} value only 4-fold higher than the substrate concentration used in the assay [IC_{50}: 20 μM, (cAMP): 5 μM)]. Thus, propentofylline inhibited with good efficiency the three PDE isoenzymes which are predominant in brain tissue, namely the calcium plus calmodulin-dependent PDE,

the cGMP-stimulated PDE and the rolipram-inhibited PDE [38, 39]. This might explain some of its pharmacological properties such as learning and memory improvement as well as its potential for the treatment of neurodegenerative diseases. Although non-selective PDE inhibitors are currently thought to be unsuitable as therapeutic agents due to their numerous side-effects, recent studies have pointed out the potential interest of mixed inhibitors, especially in the cardiovascular system [40]. For example, it has been suggested that in order to increase contractile force, it is necessary to inhibit a sufficient percentage of the total cAMP-hydrolysing activity, i.e. PDE III plus PDE IV [41].

Among the xanthine derivatives known to increase blood supply to skeletal muscles, torbafylline possesses a peculiar profile which distinguishes it, in experimental models, from other drugs such as pentoxifylline [4]. Only torbafylline was able to increase the proportion of oxidative fibers and to accentuate the aerobic energy production in muscles [42]. Increased oxidative capacity improved, in turn, muscle performance. In the present study, torbafylline proved to be the weakest PDE inhibitor and did not exhibit a marked selectivity, except for a slight preference toward the calcium plus calmodulin-dependent PDE I. Thus, it is very unlikely that its PDE inhibiting properties might be involved in its peculiar pharmacological profile.

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