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Theophylline-induced potentiation of the antinociceptive action of baclofen

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1 Theophylline (35, 50 mg/kg) potentiated the antinociceptive action of intraperitoneally administered baclofen in the tail flick and hot plate tests. Potentiation was most marked when the pretreatment time was 1 h, but some potentiation was still apparent following a 2 h pretreatment.

2 Theophylline alone (50 mg/kg) produced only slight alterations in reaction latency in the two tests.

3 When baclofen was applied directly into the spinal subarachnoid space, a 1 h pretreatment with theophylline produced minimal effects, but a 2 h pretreatment produced an increase in the antinociceptive action of baclofen.

4 These results suggest that theophylline can potentiate the antinociceptive action of baclofen by actions at both supraspinal and spinal sites.

Introduction

Baclofen produces antinociception in a variety of commonly used tests for antinociceptive activity. These include the hot plate (Cutting & Jordan, 1975; Levy & Proudfit, 1977), tail flick (Levy & Proudfit, 1977; Liebman & Pastor, 1980), writhing (Levy & Proudfit, 1977; Hill, Maurer, Buescher & Roemer, 1981), pressure (Hill *et al.*, 1981) and shock titration tests (Hill *et al.*, 1981). Antinociception occurs following microinjection into discrete brain sites in the midbrain and hindbrain (Levy & Proudfit, 1979) and administration into the spinal subarachnoid space (Wilson & Yaksh, 1978) indicating that there are both spinal and supraspinal sites of action. The suppression of the tail flick response produced by systemically administered baclofen is greatly reduced in spinally transected animals (Proudfit & Levy, 1978), suggesting that the supraspinal site is the most sensitive to the action of baclofen.

The neurochemical substrates with which baclofen interacts at these sites to produce antinociception have not been clearly defined although baclofen is known to interact with a number of neurotransmitter systems (Cutting & Jordan, 1980). Recently, studies in this laboratory indicated that alterations in catecholamine function at supraspinal sites may be involved in this action (Sawynok, 1982). Theophylline has been shown to increase noradrenaline turnover in rat brain (Berkowitz, Tarver & Spector, 1970; Karasawa, Furukawa, Yoshida & Shimizu, 1976) and to antagonize the antinociceptive action of morphine in the tail flick test (Ho, Loh & Way, 1973; Jurna, 1981). In the present study, the effects of theophylline on the antinociceptive action of baclofen were investigated in an attempt to gain further insight into the mechanism(s) by which baclofen produces antinociception.

Methods

Male Sprague Dawley rats (150–250 g i.p. experiments, 250–350 g i.t. experiments) were used. Animals were obtained from Charles River, Canada Ltd., and allowed at least 2 days acclimatization before testing. They were housed in pairs (i.p. rats) or individually (i.t. rats after cannulation) with free access to food and water on a 12/12 h light-dark cycle at 22 ± 1°C. Antinociception was evaluated using the tail flick (D'Amour & Smith, 1941) and hot plate tests (Woolf & MacDonald, 1944). Baseline latencies in the tail flick test were set at 2–3 s and a 10 s cut off imposed. In the hot plate test (hot plate maintained at 50 ± 0.5°C), baseline latencies to hind paw lick were between 5 and 15 s and a 90 s cut off was used. Following the determination of baseline latencies in the two tests (the mean of two determinations in the tail flick test followed by the hot plate test), rats were injected intraperitoneally (i.p.) (1 ml/200 g body weight) and reaction latencies determined at 30 or 45 min intervals for 2–3 h. (±)-Baclofen was used in all experiments.

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In other experiments, baclofen was injected intrathecally (i.t.) in 15 μl of saline and the catheter (volume 6–8 μl) cleared by injection of 10 μl saline. Rats were cannulated by a modification of the method of Yaksh & Rudy (1976).

Results in Figures 1, 2 and 3 were analysed by analysis of variance and the Student-Newman-Keuls test used for post-hoc comparisons.

(±)-Baclofen was supplied by Ciba-Geigy, while theophylline and caffeine were purchased from Sigma. All drugs were dissolved in 0.9% w/v NaCl solution (saline) before administration.

Results

Baclofen produced dose-related antinociception in both the tail flick and hot plate tests when applied intraperitoneally (i.p.) (5–10 mg/kg) or intrathecally (i.t.) (0.3–1.0 μg). Pretreatment with theophylline for 1 h potentiated both the peak increase in reaction latency and the duration of action in both tests when baclofen was administered i.p. (Figure 1). This effect was particularly marked following pretreatment with the 50 mg/kg dose. A similar marked potentiation was observed following pretreatment with caffeine (50 mg/kg, i.p.) (Table 1). Theophylline (50 mg/kg) itself produced minimal alterations in reaction latency when administered alone. There was a slight but significant decrease in reaction latency in the tail flick test 1 h after injection (3.0 ± 0.2 s reduced to 2.6 ± 0.1 s, $P<0.01$ in paired Student's t test, while corresponding values in saline-treated animals were 3.2 ± 0.1 and 3.1 ± 0.2 s, $n=10$ in each group) and a transient increase in reaction latency in the hot plate test 30 min after injection (8 ± 1 s increased to 24 ± 6 s, $P<0.05$ in paired Student's t test with corresponding values in saline-treated animals of 11 ± 2 and 15 ± 2 s, $n=10$). When theophylline was injected

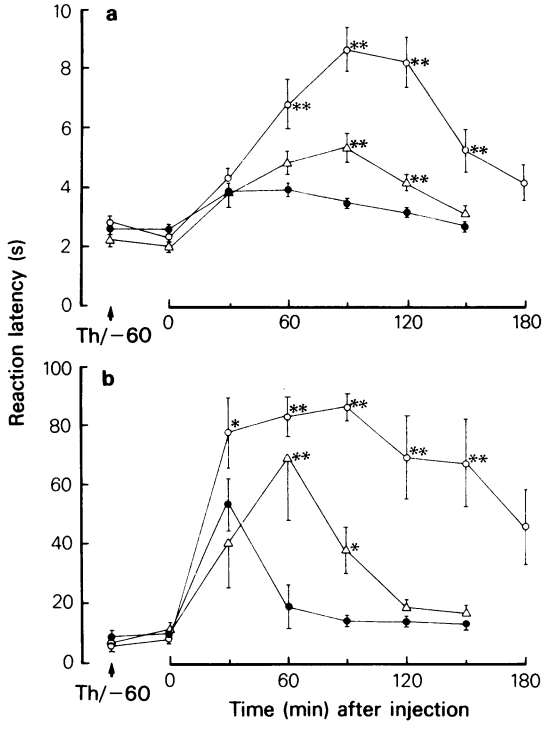

Figure 1 Effect of theophylline on the antinociceptive action of baclofen in the tail flick (a) and hot plate tests (b). All animals were injected with baclofen (5 mg/kg, i.p.) at time zero following pretreatment with saline (●), theophylline (Th) 35 mg/kg (Δ) and 50 mg/kg (o); $n=12$, 6 and 6 respectively. * $P<0.05$, ** $P<0.01$ (analysis of variance) and $P<0.05$ (Student-Newman-Keuls test) compared to saline-treated controls in individual experiments. Vertical bars indicate 1 s.e. mean.

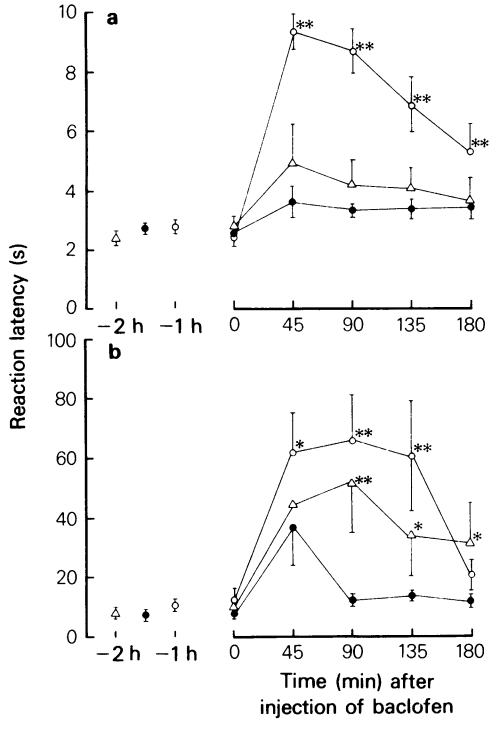

Figure 2 Influence of different pretreatment times on the effect of theophylline (50 mg/kg i.p.) on baclofen-induced antinociception in the tail flick (a) and hot plate test (b). All animals were injected with baclofen (5 mg/kg i.p.) following pretreatment with saline for 90 min (●) and theophylline for 60 min (O) or 120 min (Δ); $n=5$ in each group. *$P<0.05$; **$P<0.01$ (analysis of variance) and $P<0.05$ (Student-Newman-Keuls test) compared to saline pretreated group at same time interval.

Table 1 Effect of caffeine (50 mg/kg, i.p.) on the antinociceptive action of baclofen (10 mg/kg, i.p.)

	Time after injection of baclofen (min)					
	−60	*0*	*45*	*90*	*135*	*180*
Tail flick test						
Saline (6)	2.8±0.2	3.2±0.3	6.0±1.2	4.3±1.4	3.0±0.3	3.4±0.4
Caffeine (6)	2.5±0.3	2.9±0.4	9.6±0.4**	9.4±0.4**	9.7±0.2**	9.2±0.4**
Hot plate test						
Saline (6)	9±1	10±2	80±10	38±18	21±8	12±1
Caffeine (6)	6±1	9±2	c.o.	c.o.**	c.o.**	c.o.**

Tabulated values indicate mean±s.e. mean (s) of the latency to tail flick or hind paw lick in respective tests. c.o. indicates all animals in the group reached the cut off. Number of rats in groups indicated in parentheses. **$P<0.01$ (analysis of variance) and <0.05 (Student-Newman-Keuls test) compared to saline- and baclofen-treated group at same time interval.

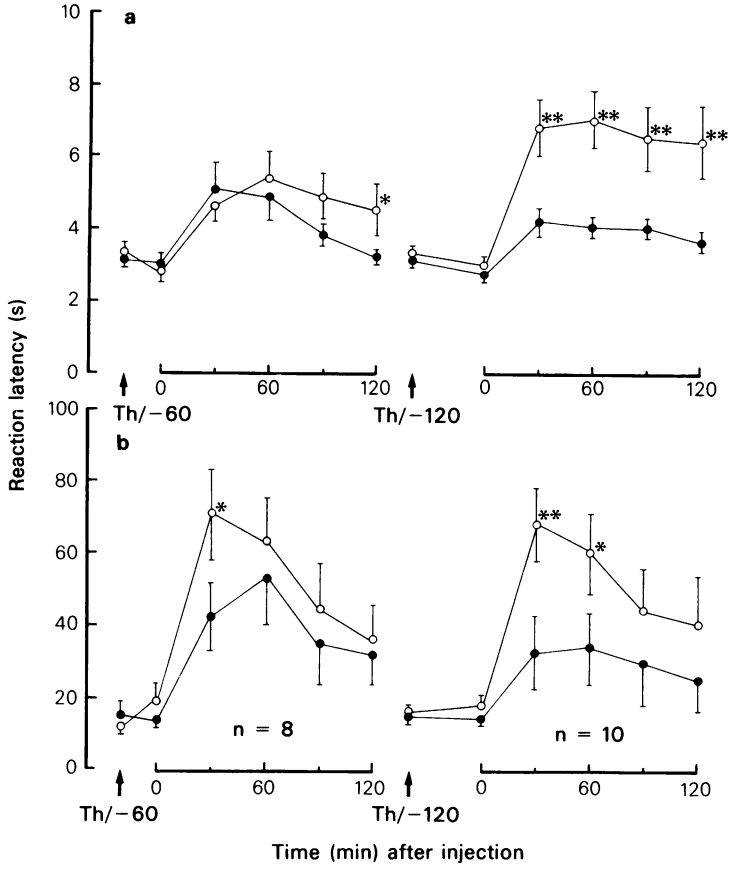

Figure 3 Effect of theophylline (Th, 50 mg/kg i.p.) on the antinociceptive action of baclofen (0.7 μg) applied intrathecally at time zero in the tail flick (a) and hot plate test (b). In the left panels, theophylline was injected 60 min before baclofen ($n=8$) while in the right panel, the pretreatment time was 120 min ($n=10$). (●) Saline pretreated groups; (○) theophylline pretreated groups. *$P<0.05$; **$P<0.01$ determined as in Figure 2.

2 h before baclofen, no potentiation in the tail flick test was observed, although some was still apparent in the hot plate test (Figure 2). This is consistent with the greater sensitivity of the hot plate test in detecting antinociceptive activity of i.p. baclofen. At the peak of baclofen's action (30–60 min after injection) tail flick latencies were evelvated to 150% of predrug values, while corresponding hot plate latencies were increased to 300–500% of predrug values.

When baclofen was administered i.t., pretreatment with theophylline for 1 h produced minimal effects on the action of baclofen with a slight delayed effect in the tail flick test and an initial effect in the hot plate test (Figure 3). However, when the pretreatment time was increased to 2 h, there was a reproducible increase in the antinociceptive action of baclofen in both tests (Figure 3).

Discussion

The present study demonstrates that theophylline produces a marked potentiation of the increase in reaction latency induced by baclofen in the tail flick and hot plate tests, two commonly used tests for antinociceptive activity. The potentiation is most marked when baclofen is administered i.p. and theophylline is given 1 h before baclofen. When baclofen is administered i.t., the same pretreatment produces minimal alterations in the action of baclofen indicating that those actions of theophylline which are critical to the potentiation probably occur at supraspinal sites. The potentiating effect of theophylline is less pronounced when it is given 2 h before i.p. baclofen. However, when baclofen is given i.t., potentiation of the spinal action of baclofen is observed. Thus, there appear to be two mechanisms by which theophylline can potentiate the action of baclofen: one at a supraspinal site with a shorter latency and another at the spinal level with a longer latency. These mechanisms probably differ because the spinal and supraspinal mechanisms by which baclofen produces antinociception probably differ even though they have not yet been clearly defined. Baclofen interacts with a number of neurotransmitter systems (Cutting & Jordan, 1980) but evidence for the involvement of individual interactions in the antinociceptive action of baclofen is not available.

In addition to producing antinociception, baclofen is known to produce motor incoordination (Levy & Proudfit, 1977)). However, antinociception can occur in the absence of motor impairment with lower doses of baclofen (Sawynok & LaBella, 1982). Conversely, motor impairment can be observed without antinociception because the motor effect has a faster onset than antinociception (Levy & Proudfit, 1977) while motor impairment occurs in the absence of antinociception following the microinjection of baclofen into discrete brainstem sites (Levy & Proudfit, 1979). A similar dissociation is observed with other drugs, for diazepam and phenobarbitone produce equivalent degrees of motor impairment to baclofen but lack antinociceptive activity (Levy & Proudfit, 1977). It is therefore unlikely that the observed effects of theophylline and caffeine on the antinociceptive action of baclofen could be due to alterations in motor function.

Theophylline has a number of pharmacological properties including noradrenaline release (Berkowitz *et al.*, 1970; Karasawa *et al.*, 1976), adenosine receptor antagonism (Daly, Bruns & Snyder, 1981), release of Ca^{2+} (Johnson & Inesi, 1969) and phosphodiesterase inhibition (Amer & Kreighbaum, 1975). Recently, it was demonstrated that agents which decrease catecholamine function (depletors of monoamines and catecholamines, noradrenoceptor and dopamine receptor antagonists) increase the antinociceptive action of baclofen, and that this effect was likely to occur at a supraspinal site (Sawynok, 1982). This observation suggests that baclofen may act to inhibit noradrenergic and/or dopaminergic systems although the anatomical location of such catecholamine systems remains to be defined. Theophylline alters the antinociceptive action of baclofen in a manner similar to agents which deplete or antagonize catecholamines. However, theophylline increases the turnover of noradrenaline (Berkowitz *et al.*, 1970; Waldeck, 1971; Karasawa *et al.*, 1976) suggesting that this action may not account for the observed potentiation. The effects of theophylline on dopamine turnover are less clear because both an increase (Waldeck, 1971) and little or no effect (Karasawa *et al.*, 1976) have been reported, but even if increases do occur, this change is again in the opposite direction to that required to account for observed changes. Alterations in catacholamine function do not appear to account for the action of theophylline, although it is possible that the determination of monoamine turnover in whole brain or very loosely defined brain regions may mask changes in a specific neuronal system critical to the antinociceptive action of baclofen.

With respect to other properties of theophylline, adenosine and related purines applied centrally produce antinociception which is blocked by theophylline (Yarbrough & McGuffin-Clineschmidt, 1981), so that interactions of theophylline with adenosine receptors would be expected to produce decreases rather than increases in antinociceptive activity. With respect to mobilization of Ca^{2+} stores and phosphodiesterase inhibition, it is difficult to assess the involvement of these actions in the observed potentiation because Ca^{2+} and cyclic adenosine 3'5'-monophosphate (cyclic AMP) have not been impli-

cated in the antinociceptive action of baclofen. In the spinal cord, Ca^{2+} (as $CaCl_2$, 50 μg i.t. injected simultaneously with baclofen) does not alter the effects of i.t. baclofen on reaction latency in either test (data not shown) indicating that the spinal mechanism is probably independent of Ca^{2+}. The mechanism(s) by which theophylline potentiates the actions of baclofen are unclear at present.

Theophylline alone produces only slight effects on reaction latencies in the two tests, decreasing the latency in the tail flick test and increasing it in the hot plate test. The opposing directions of these changes indicates that the tests are not equivalent measures of nociceptive activity, an observation supported by the differing sensitivity of the two tests to baclofen (Levy & Proudfit, 1977). Previously, both theophylline and caffeine were shown to reduce the nociceptive threshold in all measures of the vocalization test (Paalzow & Paalzow, 1973), but the mechanisms underlying changes in nociceptive threshold have not been determined.

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