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BRIEF COMMUNICATION

Clonidine Reverses Methylxanthine-Induced Potentiation of Baclofen Antinociception

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STEARDO, L. AND J. SAWYNOK. *Clonidine reverses methylxanthine-induced potentiation of baclofen antinociception.* PHARMACOL BIOCHEM BEHAV 22(5) 905–907, 1985.—The effect of clonidine on the antinociceptive effect of methylxanthine/baclofen and dopamine antagonist/baclofen combinations was examined to determine if alterations in noradrenaline turnover might mediate the potentiating effect of these agents. Clonidine alone had intrinsic activity in the tail flick test, so a dose and treatment schedule which produced a plateau effect was chosen. Clonidine pretreatment did not significantly alter the effect of baclofen alone, but reversed the potentiation of the action of baclofen produced by both theophylline and isobutylmethylxanthine. The intrinsic effect of isobutylmethylxanthine also was reversed. Combinations of dopamine antagonists and baclofen were potentiated or unaffected by clonidine. A possible interpretation of these results is that mutual interactions by baclofen and methylxanthines with descending noradrenergic pathways mediate the methylxanthine-induced potentiation of the antinociceptive effect of baclofen. A more specific determination of noradrenergic pathways involved in the action of baclofen will require the use of more specific alternative approaches.

Antinociception Clonidine Baclofen Methylxanthines Dopamine antagonists

METHYLXANTHINES potentiate the antinociceptive effect of baclofen by a predominantly supraspinal action [8] but the mechanism by which this potentiation occurs is not readily apparent. Methylxanthines have a number of pharmacological properties, but there are reasons to exclude or doubt the involvement of adenosine receptor antagonism, phosphodiesterase inhibition and calcium mobilization in this particular effect [8]. One possible mechanism is by a mutual interaction with central noradrenergic pathways. Both supraspinal and descending noradrenergic systems are implicated in the antinociceptive action of baclofen. Depletion of catecholamines with systemic reserpine and α-methyl-p-tyrosine and receptor blockade with phentolamine increases the effect of baclofen [9]. However, the more limited intrathecal application of 6-hydroxydopamine and phentolamine inhibits the antinociceptive of baclofen suggesting the above interactions reflect a supraspinal interaction [10]. Methylxanthines increase central noradrenaline (NA) turnover [1, 4, 6] perhaps by stimulating the firing of neurons in the locus coeruleus [5]. Both the increase in noradrenaline turnover [4] and activation of neuronal activity [5] are blocked by clonidine, an alpha-2 adrenergic agonist. In the present study, the effect of clonidine on the methylxanthine-baclofen interaction was examined in order to determine whether interactions with noradrenergic systems could account for the observed potentiation of baclofen antinociception.

METHOD

Sprague-Dawley rats (male, 325–400 g) were used in all experiments. Animals were housed in pairs on a 12/12 hour cycle and given free access to food and water. Antinociception was assessed using the tail flick test (baseline latency 2–3 sec, cutoff 10 sec). Animals were used for 2–3 experiments, with at least 3 days elapsing between trials. All drugs were injected intraperitoneally (IP). Baclofen, theophylline, clonidine and chlorpromazine were dissolved in saline, isobutylmethylxanthine (IBMX) was dissolved in 50/50 v/v propylene glycol/saline and haloperidol in 1.5% tartaric acid. Appropriate control vehicle injections were made where necessary. Theophylline, IBMX, chlorpromazine and clonidine were injected 60 min prior to baclofen, while haloperidol was injected immediately prior to baclofen in accordance with doses and pretreatment schedules shown previously or in trial experiments to be effective [8,9]. Statistics were calculated using ANOVA followed by the Student-Newman-Keuls (SNK) test.

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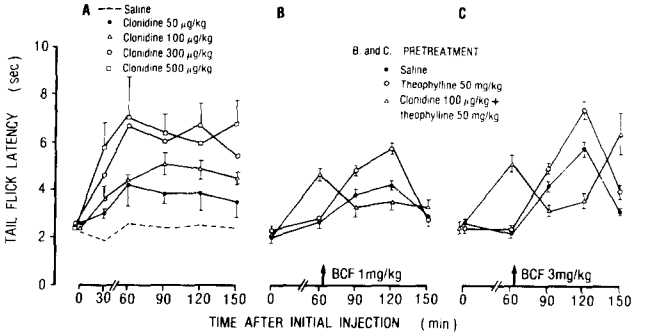

FIG. 1. Effect of clonidine on (A) tail flick latency and (B,C) antinociceptive effect of baclofen/theophylline combinations. Baclofen (BCF) 1 mg/kg (B) and 3 mg/kg (C) injected at arrows, other treatments were injected 60 min prior to baclofen after the initial baseline determination. Values are mean±SEM. In panel A, n=4 except for clonidine 100 μg/kg where n=11, in B, n=5 and in C, n=10 per group.

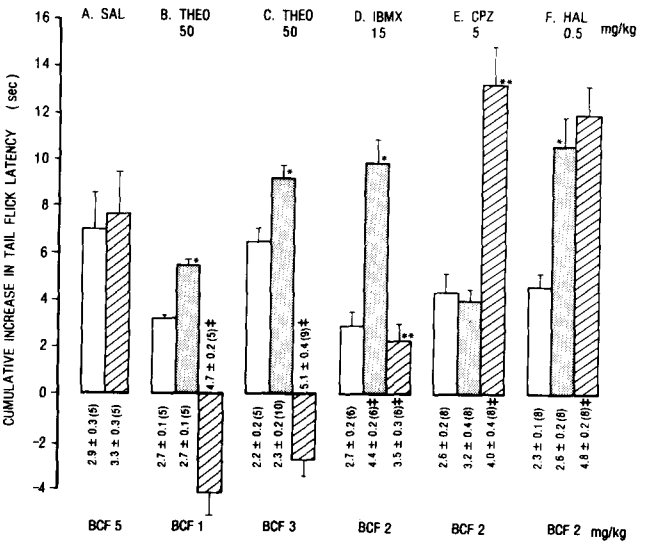

FIG. 2. Effect of clonidine pretreatment (100 μg/kg, 60 minutes) on increase in latency produced by saline (SAL), theophylline (THEO), isobutylmethylxanthine (IBMX), chlorpromazine (CPZ) and haloperidol (HAL) combinations with baclofen. Ordinate represents the cumulative increase in latency 30, 60 and 90 minutes after the injection of baclofen; negative values indicate decreases in latency (see Fig. 1B and C). Figures under columns indicate tail flick latency prior to the baclofen injection with the number of animals per group in parentheses. Open box: Saline + baclofen; dotted box: methylxanthine or dopamine antagonist + baclofen; striped box: corresponding clonidine pretreated group. *$p<0.05$ compared to baclofen group, **$p<0.05$ compared to baclofen combination group, ‡$p<0.05$ compared to corresponding value in saline or vehicle group (ANOVA and SNK test).

RESULTS

Clonidine alone produced dose-related increases in tail flick latency when tested over a 150 min time course following IP injection (Fig. 1A). Once the maximal effect was attained, this increase was sustained for at least 90 min. A ceiling effect was obtained with 300 μg/kg, with a further increase to 500 μg/kg not producing any greater increase in latency. (The ceiling effect of clonidine in this test has been noted previously, see [3]). On the basis of the dose-response relationship, a moderately effective dose of clonidine and a time course which produced a plateau effect were selected to determine interactions with other agents. Pretreatment with clonidine for 60 min did not alter the effect of baclofen administered alone (Fig. 2A). However, such pretreatment markedly reduced the effect of combinations of baclofen and theophylline, with post-injection values falling even below the effect of clonidine itself (Fig. 1B and C, Fig. 2B and C). A reduction in the effect of baclofen/theophylline combinations was obtained also with clonidine injected 120 min prior to baclofen although in this case, antagonism was observed only 30 min after the injection of baclofen (data not shown). This observation combined with data shown in Fig. 1C suggests the antagonistic effect of clonidine lasts only 2–2½ hours after its injection.

Pretreatment with clonidine also reduced the antinociceptive effect of isobutylmethylxanthine (IBMX)/baclofen combinations (Fig. 2D). In this case, experiments are more difficult to interpret because IBMX had intrinsic activity in the tail flick test, increasing latencies from 2.7±0.2 sec to 4.4±0.2 sec (n=6, $p<0.05$) 60 min after injection. Clonidine also increased latencies generally to between 4 and 5 sec (Fig. 1A, latencies under clonidine columns in Fig. 2) such that the effect of the combination is less than either individual effect. This was confirmed in a separate experiment where the respective latencies at 60 min were IBMX 5.9±1.1, clonidine 5.2±0.5 and IBMX plus clonidine 3.5±0.3 ($p<0.05$ compared to both groups, n=6–7). The potentiating effect of IBMX on baclofen was markedly reduced following clonidine pretreatment (Fig. 2D).

The specificity of the clonidine-induced antagonism of methylxanthine action was examined by a comparison with effects on chlorpromazine and haloperidol combinations with baclofen. Both agents previously were shown to increase the effect of baclofen [9]. Clonidine pretreatment did not reduce the effect of these combinations (Fig. 2E and F). With chlorpromazine there was potentiation of the effect of the combination, although with haloperidol, the increase was masked by the attainment of cutoff values in 5 of 8 rats. Curiously, chlorpromazine did not increase the effect of baclofen in these experiments (different pretreatment doses and time courses were not investigated), but this is perhaps fortuitous because it allowed the clear expression of a potentiated response in the presence of clonidine.

DISCUSSION

Methylxanthines increase the antinociceptive effect of baclofen by a predominantly supraspinal and unknown mechanism [8]. The present study was undertaken to determine if changes in NA turnover could be involved in this action. Methylxanthines increase the firing rate of NA neurons in the locus coeruleus [5] and the turnover of NA in brain regions innervated by this region [4] although the mechanism of such changes is not clear [4,5]. Both effects are reversed by clonidine, actions presumably mediated by activation of alpha-2 autoreceptors on noradrenergic cell bodies [2]. The present results indicate that pretreatment with clonidine reversed the potentiation of baclofen produced by both theophylline and IBMX suggesting that this mechanism may be relevant to the observed behavioural effects. Reversal did not appear to be non-specific in that potentiation produced by a dopamine antagonist was not af-

fected in the same manner. Although chlorpromazine and haloperidol also increase the firing rate of neurons in the locus coeruleus under some conditions [7], the lack of reversal of their effect by clonidine suggests that this action is not a major determinant of their effect on baclofen.

There are difficulties inherent in the interpretation of the present results because of the use of multiple drug treatments, particularly when more than one agent has intrinsic activity in the tail flick test. An attempt to circumvent this problem was made by using conditions under which the intrinsic effect of clonidine approximated a plateau value, but values falling below this level were recorded. The IBMX experiments are particularly complicated to interpret because three agents have intrinsic activity, and clonidine appeared to reverse both the effect of IBMX and the IBMX/baclofen combination. Although IBMX and theophylline are both methylxanthines and exert a qualitatively similar effect on NA turnover, the effect of IBMX is much more pronounced [4], and the behavioural effects elicited by these agents differ. Theophylline produces restlessness and agitation while IBMX produces mainly lethargy, ptosis and "bloody tears," in agreement with earlier reports [4,11]. Similarly, in the tail flick test, theophylline did not alter reaction latencies, while IBMX produced a significant increase in latency (Fig. 2).

Baclofen appears to interact with central NA systems in a complex manner. The intraperitoneal administration of monoamine depleting agents and receptor antagonists potentiates the effect of intraperitoneal but not intrathecal baclofen suggesting baclofen diminishes supraspinal NA function [9], while more specific depletion of descending NA pathways with intrathecal 6-hydroxydopamine as well as intrathecal amine antagonists inhibits the effect of baclofen suggesting an activation of descending pathways [10]. An enhanced turnover in supraspinal NA pathways by methylxanthines would tend to diminish the effect of baclofen, but an enhanced turnover in spinal NA pathways would tend to reinforce this effect. Behaviourally, potentiation is observed suggesting the descending pathway exerts a predominant action. If clonidine diminishes the activity of NA neurons in both supraspinal and spinal pathways but the spinal pathway is predominant, a reversal of potentiation would occur. Perhaps in the case of a combination of clonidine and baclofen, the net effect of clonidine on ascending and descending pathways is a cancellation such that behaviourally, no effect is observed. A further delineation of the specific NA pathways involved in the antinociceptive effect of baclofen will require simultaneous measurement of supraspinal and spinal NA turnover following pretreatment with baclofen and methylxanthines.

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