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Possible Purinergic Mediation of Anxiolytic Effect of Carbamazepine Measured in the Conflict Test with Rats

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SUMMARY. Using a modified Geller and Seifter test in rats the mechanisms of anticonflict effect of carbamazepine was investigated. The purinoceptor antagonist aminophyline (5 mg/kg) blocked the carbamazepine effect in this paradigm. On the other hand, papaverine (5 mg/kg) significantly increased the punished responses, as observed with anxiolytic drugs which indicates an anticonflict effect for this drug. Carbamazepine (2.5 mg/kg) and papaverine (2.5 mg/kg) when administered in combination with doses which had no effects by themselves, showed statistically significant increase on punished responding. Therefore, these results provide preliminary evidence suggesting that carbamazepine acts in the anticonflict effect through its action on purinoceptors.

RESUMEN. "Posible Mediación Purinérgica del Efecto Ansiolítico de Carbamazepina medida por medio del Test de Conflicto en Ratas". Se investigó en ratas el mecanismo del efecto "anticonflicto" de la carbamazepina, empleando una pueba de Geller y Seifter modificada. El antagonista purino-receptor aminofilina (5 mg/kg) bloqueó en este paradigma el efecto de la carbamazepina. Por otra parte la papaverina (5 mg/kg) aumentó significativamente las respuestas al castigo, como se observa con los fármacos ansiolíticos, lo que muestra su efecto "anticonflicto". Cuando se administraron la carbamazepina y la papaverina en combinación, a dosis de 2,5 mg/kg, respectivamente, se observó un aumento significativo en la respuesta al castigo. Los resultados proporcionan evidencia preliminar de que la carbamazepina actúa a través de su acción sobre los purino-receptores.

INTRODUCTION

Although classified as an antiepileptic agent, used in the treatment of complex partial epilepsy and generalized tonic-clonic seizures, carbamazepine (CBZ) is also effective in two animal models of anxiety, which are predictive of clinical anxiolytic drug action [1,2].

Adenosine or related nucleotides may present sedative and anticonvulsant properties when administered peripherally or centrally to mammals [3-5]. This purine has also hypnogenic properties and when given at high doses it presents anti-nociceptive action [6]. *In vitro,* adenosine agonists are potent modulators of adenylate cyclase activity and of neurotransmitter release [7]. In general, these effects of adenosine can be antagonized by the alkylxanthine compounds such as caffeine, theophylline and aminophylline (AMN), which could act as adenosine-receptor antagonists in the central nervous system [8].

A previous study, showed that CBZ reduced the effect of 1-methyl-isoguanosine, an adenosine agonist, in the isolated guinea pig ileum. It was also demonstrated that theophylline, an adenosine antagonist, significantly decreased the anticonvulsant effect of CBZ. These results suggest that the pharmacological effect of CBZ could be the result of interference of this drug in the adenosine-mediated neurotransmission [9,10].

In the present study we evaluated whether the anticonflict effect of CBZ may be affected by

KEY WORDS: Adenosine-mediated neurotransmission, Aminophylline, Anxiolytic activity, Carbamazepine, Conflict-test, Papaverine.
PALABRAS CLAVE: Actividad ansiolítica, Adenosina, Aminofilina, Carbamazepina, Papaverina, Test del conflicto.

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aminophylline, an antagonist of adenosine receptors, or by papaverine, an inhibitor of adenosine neuronal uptake.

MATERIALS AND METHODS

Animals

Twenty male Wistar rats, 90-120 days old, were used. They were acclimatized to the laboratory conditions preceeding the experiment. They were housed in groups of two or three in wire mesh cages with free access to food and kept on a 12-hour light cycle and at constant temperature (22 °C ± 2 °C).

Drugs

The following drugs were used: carbamazepine (CBZ, Ciba-Geigy-Brazil); aminophylline (AMN, Sandoz-Brazil); papaverine (PAP, Veado d'ouro- Brazil).

Drugs Administration

All drugs were injected i.p. in a constant volume of 0.1 ml/100g. CBZ was suspended in saline with tween-80, 1% v/v and were administered 1h before the test. AMN and PAP were dissolved in 0.9% w/v NaCl. Aminophylline was injected 45 min before the test. Papaverine was administered 15 min prior to the experimental session. The control animals received only the vehicle.

Apparatus

During daily experimental sessions, the rats were placed in a box (Grason-Stadler Co, model 1101) with electrifiable steel grid floor and a response lever placed to the left and above a liquid dispenser. A panel light installed above the lever allowed the presentation of a luminous stimulus. The shock generator (Albarsch do Brazil) was a solid state scrambler-shocker, which delivered a constant current adjustable from 0 to 1.0 mA.

Procedure

A modified Geller-Seifter test was employed for the studies [11]. A total of twenty rats (two groups of ten) deprived of water for 20 h were trained to press a lever for liquid reward on a fixed ration (FR-5) reinforcement schedule. Then a luminous stimulus was introduced at variable time intervals after their achieving stable response rates. This was a signal that every lever press would be reinforced [continuous reinforcement (CRF) schedule]. Five sessions later, a shock (0.7 mA, 60 cycle, AC) was delivered following each lever press during the period of luminous stimulus presentation. The session lasted 12 min each. There was at least 3 days interval between drug administration. Sessions were run during these intervals with control solution administration.

Statistical analysis

The results were expressed as means and the standard errors. The Student's t-Test was used to compare two means (Figure 2). Data were analyzed in a one-way analysis of the variance followed by Dunnett's Test. Difference were considered to be statistically significant when $p < 0.05$.

RESULTS

As shown in Figure 1, an increased number of lever presses can be seen after CBZ (20 mg/kg) during the punished period (B). Aminophylline in a dose of 5 mg/kg prevented this effect. No alteration can be noted in response rates after drug treatments during the unpunished period (A).

The effect of single doses of PAP, CBZ and AMN on the number of lever presses are presented in Table 1. An increased bar pressing can be observed with 5 and 10 mg/kg doses in the papaverine-treated group during the punished period. With these doses the mean frequency of response of the Saline and PAP group was similar during the unpunished period. A significant reduction of lever presses in unpunished or in punished period can be observed with doses of 20 and 40 mg/kg of PAP.

The dose of CBZ (2.5 mg/kg) in combination with a dose of 2.5 mg/kg of PAP was chosen since they did not alter response rates in unpunished or in punished period when administered alone. The results in figure 2B show that the combination of CBZ and PAP increased the response rates during the punished period in comparison to control. No difference in the number of lever presses can be seen after the treatment, during the unpunished period (A).

DISCUSSION

In the present study, previous results of Almeida & Leite [1] and Zangrossi *et al.* [2] on the anticonflict activity of carbamazepine were confirmed. An increase in response rates of rats submitted to a Geller & Seifter conflict model was observed after CBZ, during the punished period, as can be seen in figure 1B. A purinoceptor antagonist AMN prevented this effect without any modification in the bar pressing rate during the unpunished period.

PAP, an adenosine uptake inhibitor [10,11] pro-

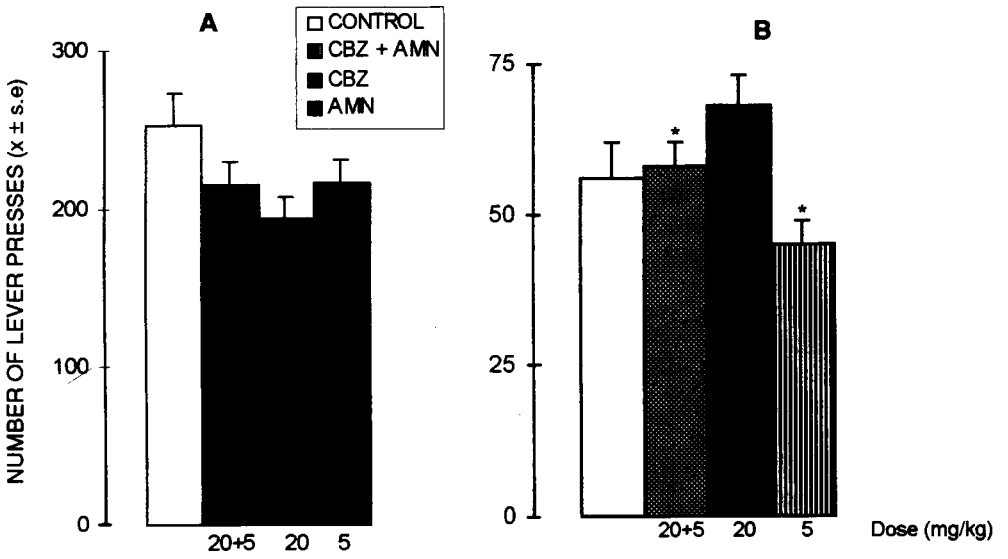

Figure 1. Effects of carbamazepine or carbamazepine plus aminophylline on conditioned suppression of behavior, represented as the number of lever pressing (mean and standard error) during the non-punished (A) or punished period (B). The doses of CBZ and AMN are shown under the columns. * Significantly different from control group. Dunnett's test, $p < 0.05$.

Treatment	Dose (mg/kg, i.p)	Number of lever Presses (x ± s.e) Non-punished	Punished
Saline	-	179.7 ± 21.4	32.6 ± 5.9
Papaverine	2.5	170.9 ± 15.2	22.2 ± 7.8
	5.0	176.4 ± 12.1	39.0 ± 2.1*
	10.0	174.3 ± 15.6	56.6 ± 2.8*
	20.0	130.4 ± 14.5*	28.0 ± 5.7
	40.0	36.8 ± 11.3*	8.0 ± 3.9*
Saline	-	181.5 ± 14.3	23.5 ± 4.3
Aminophylline	2.5	179.1 ± 12.7	20.2 ± 2.3
	5.0	170.7 ± 9.9	13.1 ± 3.2*
	20.0	167.6 ± 11.6	11.7 ± 4.8*
Vehicle	-	195.7 ± 17.4	27.4 ± 5.4
Carbamazepine	2.5	189.4 ± 12.3	23.2 ± 4.3
	5.0	201.5 ± 15.5	36.4 ± 3.1*
	10.0	210.3 ± 13.8	43.6 ± 3.7*
	20.0	198.4 ± 7.6	90.5 ± 3.9*

Table 1. Effect of papaverine, aminophylline and carbamazepine on conditioned suppression of behavior, represented as the number of lever pressing responses during a non-punished or a punished period.
* Differs significantly from the respective control group.

duced an apparent dose-dependent depressant effect. Nevertheless, an increase in punished response was observed with the doses of 5 and 10 mg/kg which may indicate a possible anticonflict effect for this drug. This result confirms those obtained by Zangrossi *et al.* [2] using the elevated-plus maze model to test antianxiety effect of a drug. The combination of a non-effective dose of CBZ and PAP also significantly increased the punished response without altering bar pressing during unpunished period.

Previous reports presented evidence that CBZ affects several neurotransmission pathways that may be involved in the control of anxiety. Among the neurotransmitters, CBZ affects the serotonergic [13,14], the GABAergic [15-18] and the NMDA excitatory amino acid system [19]. Considering previous results on the adenosinergic effect of carbamazepine and the results presented in this paper, the anticonflict effect of carbamazepine could involve an interaction of this drug with the adenosine-mediated neurotrans-

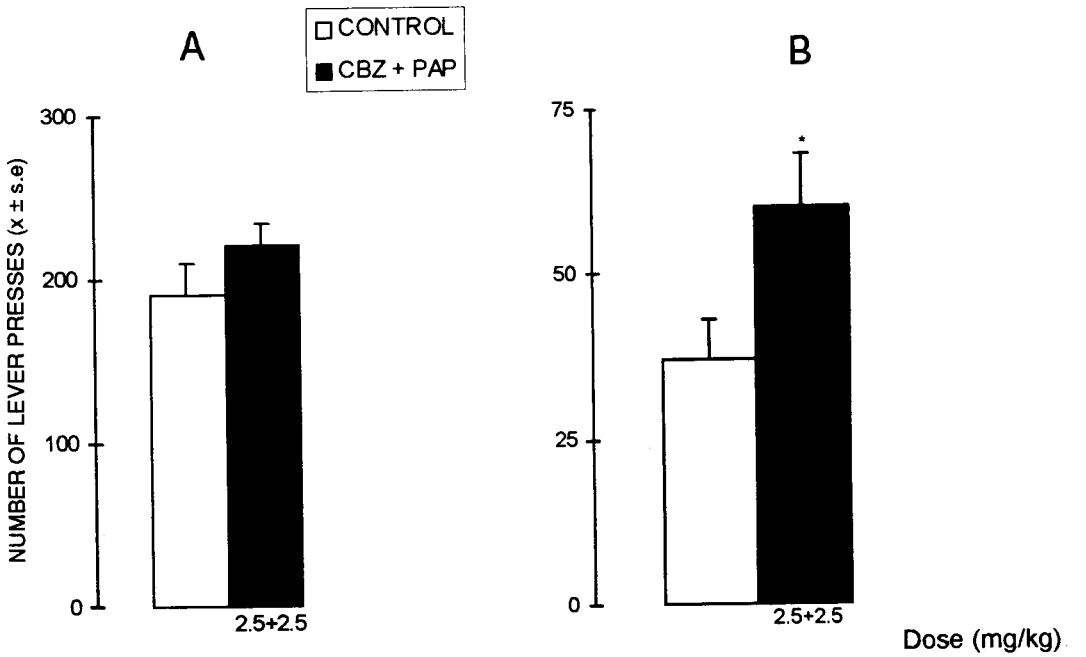

Figure 2. Effects of carbamazepine plus papaverine on conditioned suppression of behavior, represented as the number of lever pressing (mean and standard error) during the non-punished (A) or punished period (B). The doses of CBZ and PAP are shown under the columns.
* Significantly different from control group. Student's t-Test, $p < 0.05$.

mission. However this hypothesis should be taken with restriction considering that papaverine and aminophylline yield other neuronal effects besides those on the adenosinergic system. Therefore, a possible physiological or behavioral interaction as a result of the effects of these drugs should also be considered as a plausible explanation of the presented results.

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