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EFFECT OF CARBAMAZEPINE ON THE SPONTANEOUS BEATING OF ISOLATED GUINEA PIG ATRIA

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ABSTRACT

Carbamazepine, a drug effective in pain, seizure, and affective disorders, was studied for its effects and toxicity on spontaneously beating isolated guinea pig atria. Carbamazepine (20-30 μg/mL) has a negative chronotropic effect on artria, without any significant effect on contractile force. The most significant effect (12.5%) was seen with 30 μg/mL of carbamazepine on atria. With higher doses (>30 μg/mL) carbamazepine produced toxic effects which resulted in atrial standstill. Pretreament of atria with theophylline (5-50 μg/mL) prevented the negative chronotropic effect of carbamazepine (30 μg/mL). Three dose ratios of carbamazepine (1.33, 2, 2.33) in the presence of three different doses of theophylline (30, 50 and 60 μg/mL) were obtained. These results suggest that the negative chronotropic effect of carbamazepine and its toxicity may be due to its action as an agonist on adenosine A1 receptors and as an antagonist on A2 receptors of the atria. Moreover, using adenosine antagonists such as theophylline may overcome the toxic effect of carbamazepine on the heart. This may explain the reason for the interaction between carbamazepine and theophylline in clinical settings.

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Keywords: Carbamazepine, isolated atria, theophylline, adenosine receptors.

INTRODUCTION

Carbamazepine is a tricyclic anticonvulsant drug. Its most pronounced clinical effect is in seizure disorders,[6] paroxysmal pain syndromes[16] and treatment of manic-depressive disorders[1] Carbamazepine is also effective in terminating digital-induced ventricular tachyarrhythmias.[21] Carbamazepine blocks sodium channels at therapeutic concentrations and inhibits high-frequency repetitive firing in neurons in culture.[14] It has also been shown that carbamazepine in therapeutic doses can interact with adenosine receptors.[16,20] It has been suggested that the anticonvulsant properties of carbamazepine may be partially explained by an influence of this drug on adenosine receptors.[3]

been shown that carbamazepine interacts competitively with adenosine-binding sites in the brain.[23] It has been claimed that carbamazepine binds to A1 receptors completely and to A2 receptors partially at therapeutic levels.[12,8,11]

derivatives may act as agonists of A1 receptors and antagonists of A2 receptors.[12] Moreover, methylxanthines have stimulant and convulsant properties due to their action as antagonists on both A1 and A2 receptors.[12] It has been shown that theophylline completely reverses the anticonvulsant action of carbamazepine.[15] Carbamazepine also increases the severity of heart block caused by adenosine.[5]

Adenosine receptors are G-protein coupled receptors and are divided into four subtypes, including A1, A2, A3, and A4, based on agonist actions of adenosine.[13] A1 and A2 receptors are antagonized by xanthines, whereas A3 and A4 receptor are not.[13] Stimulation of A1 recep-

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tors has been associated with inhibition of adenylyl cyclase, and activation of K+ currents as well as activation of phospholipase C in some circumstances, and ion channel regulation.[13,22] A2 receptors stimulate adenylyl cyclase activity.[13,22] Activation of A3 receptors also causes the release of inflammatory mediators such as histamine from mast cells.[2] The physiologic role of A4 receptors remains unclear.[13] Adenosine has negative chronotropic and dromotropic effects in patients with paroxysmal supraventricular tachycardia.[10,17] These effects of adenosine would be mediated by A1 receptor subtypes.[9] By acting on both the sinoatrial and atrioventricular nodes, adenosine causes bradycardia and heart block.[9] Adenosine analogues have inhibitory effects in guinea pigs atria.[4] Theophylline is a nonselective adenosine receptor antagonist.[9] Theophylline and carbamazepine reduce the efficacy of each other when they are co-administered.[5]

The aim of the present study was to determine the effects of carbamazepine on isolated heart, to find a way to prevent the toxic effects of carbamazepine on the heart and to explore the interactions between carbamazepine and theophylline.

MATERIAL AND METHODS

Experimental protocol

Guinea pigs (300-700g) of either sexes were killed by a sharp blow to the back of the neck. The hearts were excised and placed in cool oxygenated Ringer Lock solution of the following composition (gram per liter): NaCl, 9; KCl, 0.42; $CaCl_2$, 0.12; $NaHCO_3$, 0.5; and glucose, 1. The atria were dissected and isolated from other tissues. The preparation was then mounted in a 20-mL organ bath which contained Ringer Lock solution, gassed with a mixture of 95% oxygen and 5% CO_2 and maintained at a constant temperature of 36-37°C. A resting tension of 1g was applied and kept constant by readjustment during the equilibration period. The mechanical activity was recorded isometrically by means of a Grass 701E polygraph. The preparation were allowed to equilibrate 30 minutes before the use of drugs. The organ bath solution was changed every 15 minutes during the equilibration period. Each time the volume of drug solution added to the organ bath was 0.1 mL . In this study all drug concentrations are indicated as milligram of drug per milliliter of organ solution. Carbamazepine was dissolved in propylene glycol to which the tisues in the organ bath were exposed (0.005 mL/mL) and had no significant effect on the rate and force of contraction of isolated atria. Theophylline was dissolved in distilled deionized water. In each experiment six guinea pigs were used.

Statistical analysis

Responses were measured as percentage of basal levels. Statistical significance was evaluated by the t-test for paired and unparied samples and P values of 0.05 or less were considered to be significant.

Drugs

Carbamazepine, theophylline and propylene glycol (Sigma, USA).

RESULTS

In order to determine the effects of carbamazepine alone on spontaneously beating isolated guinea pig atria, carbamazepine was added to the organ bath at concentrations of 2.5-40 μg/mL. At concentrations of 2.5-15 μg/mL carbamazepine had no effect on spontaneously beating isolated atria. At concentrations of 20-30 μg/mL it decreased the rate of contraction (Fig. 3), without any significant change in contractile force. At a concentration of 30 μg/mL it produced the most significant negative chronotropic effect (12.45%) on isolated atria ($p<0.025$). At higher doses (>30 μg/mL), carbamazepine produced bradyarrhythmias which lead to atrial standstill.

In order to determine the effects of theophylline alone on spontaneously beating isolated guinea pig atria, theophylline was added to the organ bath at concentrations of 2.5-60 μg/mL. Theophylline increased the rate and force of contraction of isolated atria in a dose-dependent manner (Figs. 1 and 2). Theophylline at a concentration of 50 μg/mL produced the most potent inotropic (86.64%) and chronotropic (21.15%) effects on isolated atria.

Atria were pretreated with different doses of theophylline (2.5-50 μg/mL). After an incubation period of 10 minutes, carbamazepine at a concentration of 30 μg/mL, which showed the most significant negative chronotropic effect, was added to the organ bath. Then the effects of carbamazepine (30 μg/mL) in the presence of different doses of theophylline was investigated as well. In this study theophylline prevented the negative chronotropic effect of carbamazepine (30 μg/mL) in a dose-dependent manner (Fig. 3).

Three dose ratios of carbamazepine in the presence of three different doses of theophylline were obtained. Atria were pretreated with theophylline (30 μg/mL). After an incubation period of 10 minutes, carbamazepine (20-50 μg/mL) was added. The effective dose of carbamazepine in the presence of theophylline (30 μg/mL) shifted from 30 μg/mL to 40 μg/mL and the dose ratio was 1.33. The toxic dose of carbamazepine in the presence of theophylline (30 μg/mL) shifted from 40 μg/mL to 50 μg/mL (Fig. 4). Then the atria were pretreated with theophylline (50 μg/mL) and after the incubation period, different concentrations of carbamazepine (25-

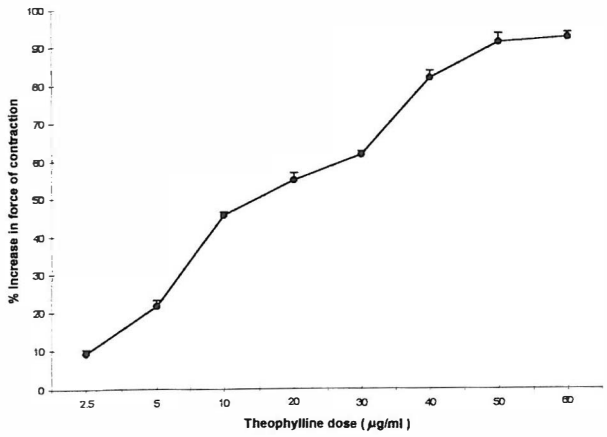

Fig. 1. Log dose-response curve of spontaneously beating isolated guinea pig atria to the inotropic effect of theophylline.

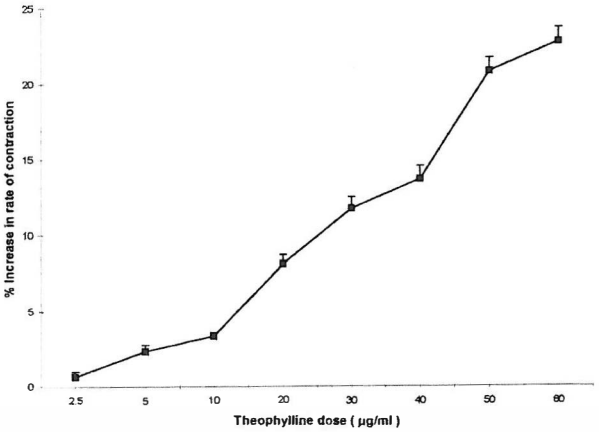

Fig. 2. Log dose-response curve of spontaneously beating isolated guinea pig atria to the chronotropic effect of theophylline.

60 μg/mL) were added. The effective dose of carbamazepine in the presence of theophylline (50 μg/mL) shifted from 30 μg/mL to 60 μg/mL and the dose ratio was 2. The toxic dose of carbamazepine in the presence of theophylline (50 μg/mL) shifted from 40 μg/mL to 70 μg/mL (Fig. 4). Finally, the atria were pretreated with theophylline (60 μg/mL) and after the incubation period, different concentrations of carbamazepine (40-80 μg/mL) were added. The effective dose of carbamazepine in the presence of theophylline (60 mg/mL) shifted from 30 μg/mL to 70 μg/mL and the dose ratio was 2.33. The toxic dose of carbamazepine in the presence of theophylline (60 μg/mL) shifted from 40 μg/mL to 80 μg/mL (Fig. 4).

DISCUSSION

It has been shown that the anticonvulsant properties of carbamazepine may be partially explained by an influence of this drug on adenosine receptors.[3,15] It has been claimed that carbamazepine interacts competitively with adenosine-binding sites in the brain.[23] It has also been shown that theophylline completely reverses the anticonvulsant action of carbamazepine.[15] It has been suggested that carbamazepine acts as an agonist on A1 receptors and an antagonist on A2 receptors.[12] In this study carbamazepine had a negative chronotropic effect on spontaneously beating isolated guinea pig atria. This is similar to the adenosine effect.[4] Pretreatment of atria with theophylline (5-50 μg/mL), an unselective antagonist of adenosine receptors, prevented the negative chronotropic effect of carbamazepine. The negative chronotropic and dromotropic effects of adenosine are mediated by A1 receptor subtypes.[9] Stimulation of A1 receptors causes inhibition of adenylyl cyclase and enhances potassium conductance[13,22] which both lead to supression of automaticity of the sinus node.[10] Stimulation of A2 receptors activates adenylyl cyclase.[13,22] Thus the antagonism effect on A2 receptors may lead to further inhibition of adenylyl cyclase which favors more suppression of the sinus node. The results may suggest that carbamazepine produces its negative chronotropic effect by acting as an agonist on A1 receptors and an antagonist on A2 receptors of isolated guinea pig atria. It is also suggested that theophylline, an unselective adenos-

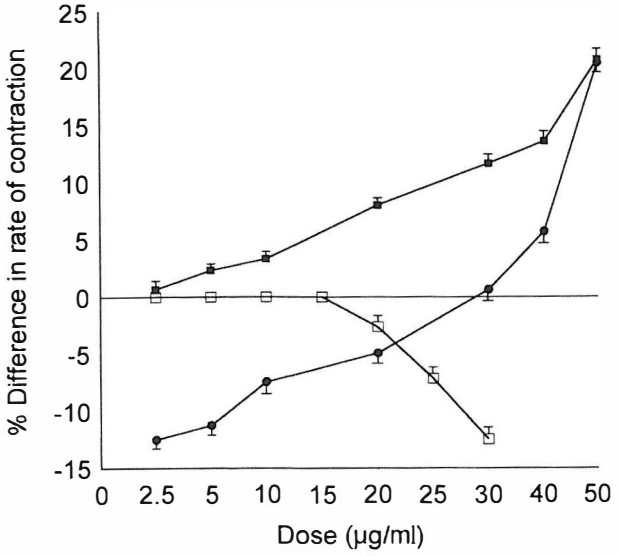

Fig. 3. Log dose-response curve of spontaneously beating isolated guinea pig atria to the chronotropic effects of (□) carbamazepine alone, (●) theophylline in the presence of carbamazepine (30 μg/mL), and (■) theophylline alone.

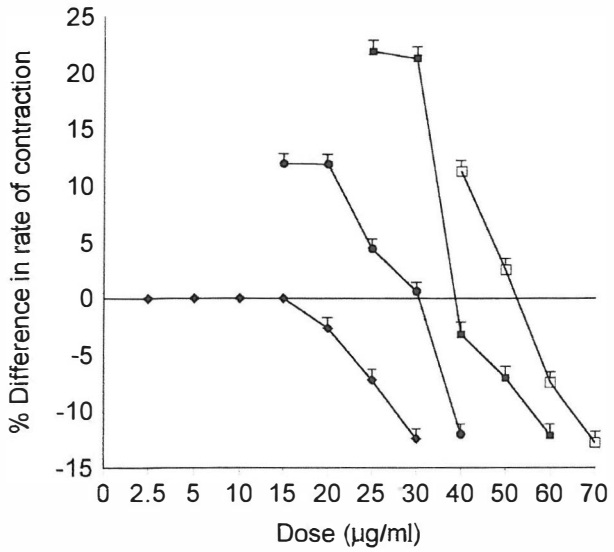

Fig. 4. Log dose-response curves of spontaneously beating isolated guinea pig atria to the chronotropic effect of (♦) carbamazepine alone. (●) carbamazepine in the presence of theophylline (30 µg/mL). (■) carbamazepine in the presence of theophylline (50 µg/mL). and (□) carbamazepine in the presence of theophylline (60 µg/mL).

ine antagonist, may compete with carbamazepine for binding to A1 and A2 receptors and inhibit the agonist effects of carbamazepine on A1 receptors. In this study, carbamazepine at higher doses (>30 µg/mL) produced toxicity in the form of bradyarrhythmias which ended as atrial standstill. It has been reported that carbamazepine moderately prolongs A-V conduction.[21] Carbamazepine intensifies the toxic effects of adenosine and may increase the degree of heart block caused by adenosine.[5] So the toxic effect of carbamazepine on isolated atria (i.e., bradyarrhythmias) may be due to its action as an agonist of A1 receptors and an antagonist of A2 receptors. In the presence of theophylline the toxic dose of carbamazepine was shifted to the right and was dependent on the theophylline dosage (Fig. 4). So we suggest that by using adenosine antagonists such as theophylline, one may overcome the toxic cardiac effect of carbamazepine. We also showed the interaction of carbamazepine with theophylline.

In the presence of different doses of theophylline, carbamazepine's curves shifted to the right (Fig. 4). Theophylline in a dose dependent manner prevented the effects of carbamazepine on isolated atria. It may be concluded that theophylline and carbamazepine compete with each other to bind to adenosine receptors in atria. The results indicate that these two drugs may reduce the efficacy of each other when they are co-administered. The results also showed that carbamazepine may have interaction with adenosine and intensify the toxic effects of adenosine when they are co-administered.

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