ORIGINAL ARTICLE

ANTAGONISM BY CAPSAICIN AND PROCAINAMIDE OF THE DEPRESSIVE EFFECT OF VERAPAMIL ON ISOLATED RAT ATRIA

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SUMMARY

The brief stimulatory effect of capsaicin (2 and 10 µg/ml) on the rate and isometric tension by isolated rat right and left atria respectively was slightly affected by a calcium antagonist, verapamil (0.05 µg/ml), whether both drugs were added simultaneously or capsaicin added 15 min after verapamil. However, in the presence of capsaicin, the negative chronotropism and inotropism induced by verapamil were markedly attenuated. Reserpine pretreatment did not abolish this antagonistic activity of capsaicin, indicating that endogenous catecholamines were not involved. Procainamide (10 µg/ml) which produced insignificant atrial stimulation was also found to mitigate the verapamil-induced atrial depression. The possible mechanism by which capsaicin and procainamide antagonize verapamil effect on isolated rat atria is discussed.

Capsaicin has well documented effect on the cardiovascular system (Virus and Gebhart, 1979). Most cardiovascular responses to capsaicin have been studied in whole animals in which capsaicin has been shown to reflexly induce either cardiovascular depression or stimulation depending on the site of administration. For instance, capsaicin injection into splanchnic (Toh et al., 1955) or hindlimb circulation (Webb-Peploe et al., 1972) elicits cardiovascular excitation while cardiovascular inhibition has been reported when capsaicin is injected into portal circulation of the liver (Ashton et al.,

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1982). The in vitro effect of capsaicin on isolated cardiac tissues, however, has received relatively little attention. In 1969, Molnar et al. and Fukuda and Fujiwara reported that low doses of capsaicin exerted the positive chronotropic and inotropic effect on isolated guinea pig atria. On the other hand, Toda et al. (1972) could not demonstrate the stimulatory effect of capsaicin on the isolated atria from rabbit and dog. The discrepancy is presumably due to the species differences. The atrial stimulation mediated by capsaicin is unaffected by propranolol or cocaine (Molnar et al., 1969) or by reserpine pretreatment (Fukuda and Fujiwara, 1969). This communication reports the effect of capsaicin on the rate and isometric force by isolated rat right and left atria respectively. The results show that capsaicin produces a brief stimulation on the rate and contractile force which is not inhibited by verapamil. However the negative chronotropic and inotropic effect of verapamil on the isolated rat atria is reduced in the presence of capsaicin as well as procainamide.

MATERIALS AND METHODS

Male Wistar rats weighing 250-300 gm were sacrificed by a blow on the head. The atria were rapidly dissected out and separated into the right and left sides. They were then mounted in organ bath chambers containing 25 ml of Locke solution gassed with 100% oxygen and maintained at 37°c by a thermoregulating water pump (Churchill Instrument Co., Ltd.) The right atria, which beat spontaneously, and the left ones were used in studying the rate and isometric force respectively. The left atria were electrically driven with square wave pulse delivered through platinum electrodes to beat at the constant rate of 250/min. The stimulus strength was 5 V and the duration was 5 msec (the stimulator used was a locally made model). The rate and contractile force were recorded with isometric force displacement transducer (Grass FT 03 C) connected to a Beckman Dynograph recorder (type R). The atria were allowed to equilibrate until stable rate and contractile force were observed before the experiments began. A preload of 1 gm was initially applied to the atria in every experiment performed.

Rats were reserpinized by intraperitoneally injected with 5 mg/kg/day reserpine (Serpasil® inj., Ciba-Geigy) for two consecutive days. On the third day, the animals were sacrificed and the atria were used in experiments.

The changes in rate and isometric tension were presented as means ± S.E.M. relative to controls. The control rate and force were determined just before drug additions and were taken as 100%. Statistical analysis was performed by unpaired Student't test; the differences were considered significant when p values were less than 0.05.

Verapamil hydrochloride (Isoptin® inj., Knoll) and procainamide hydrochloride (Pronestyl® inj., Squibb) were obtained as solution in ampuls for human injection. They were added to the bathing solution as such. Capsaicin (Sigma Chemical Co.) was dissolved in absolute ethanol; and only small volume (1-5 µl) was added. Control experiments showed that the same volume of ethanol had practically no effect on the rate and isometric tension during 30 min of experimentation.

RESULTS

Antagonism of the negative chronotropic and inotropic effect of verapamil by capsaicin

The effects of capsaicin (2 µg/ml), verapamil (0.05 µg/ml) and capsaicin added 15 min after verapamil on the spontaneous rate of isolated rat right atria are recorded in Fig. 1. Verapamil produced the well known negative chronotropic effect in which the mean reduction in rate was approximately 26% after 30 min of drug exposure. Capsaicin caused a brief stimulation of the rate which reached the peak at 1 min and then gradually declined to controls. Capsaicin still elicited the positive chronotropic response when added 15 min after verapamil. In fact, the percentage increase in rate appeared somewhat greater than controls with capsaicin alone. However, the unexpected and interesting observation was that the increased rate did not return to the pre-capsaicin value; whereas the atrial rate stimulation evoked by capsaicin alone usually subsided within 15 min. This finding indicated

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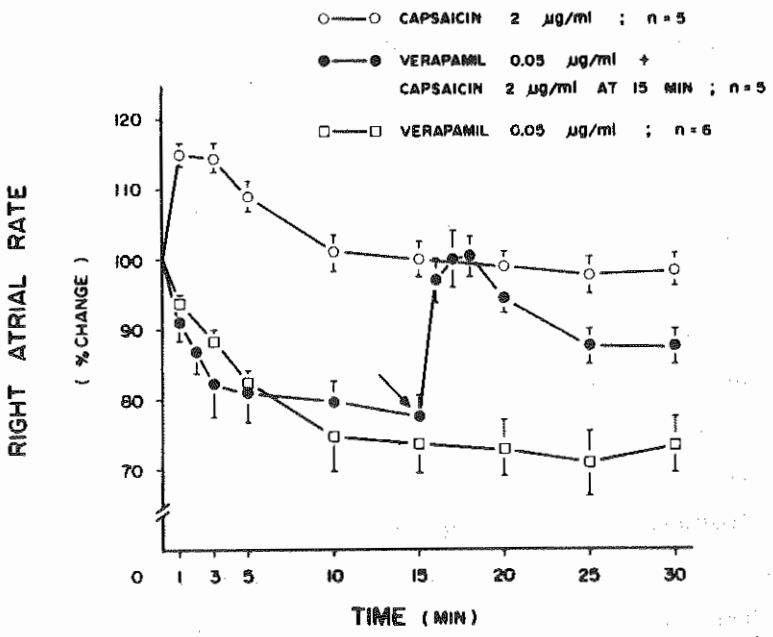

Figure 1. Lack of inhibitory effect of verapamil on the positive chronotropic response evoked by capsaicin. When both drugs were present, capsaicin was added 15 min (arrow) after verapamil.

antagonism of the verapamil-mediated negative chronotropism by capsaicin. This conclusion was confirmed in other experiments reported in Fig. 2 in which there was striking inhibition of verapamil effect when both compounds were added simultaneously. Although verapamil could not prevent the initial rate increase induced by capsaicin, it caused the augmented rate to decline faster than that observed with capsaicin alone. Other experiments not shown here revealed that reserpine pretreatment did not obliterate this antagonistic activity of capsaicin. The 30 min rate from reserpinized atria with 0.05 µg/ml verapamil was 73.93 ± 6.14% of controls, n = 5; the corresponding rate when 2 µg/ml capsaicin was also added simultaneously was 92.68 ± 3.37%, n=5 ($p < 0.05$, unpaired Student't test).

The ability of capsaicin to alleviate the negative inotropic effect of verapamil on the electrically driven isolated left atria is illustrated in Fig. 3. Capsaicin (10 µg/ml) caused transient augmentation of contractile force which was rapidly followed by depression. Lower dose (2 µg/ml) produced similar response except the augmented force fell less rapidly and

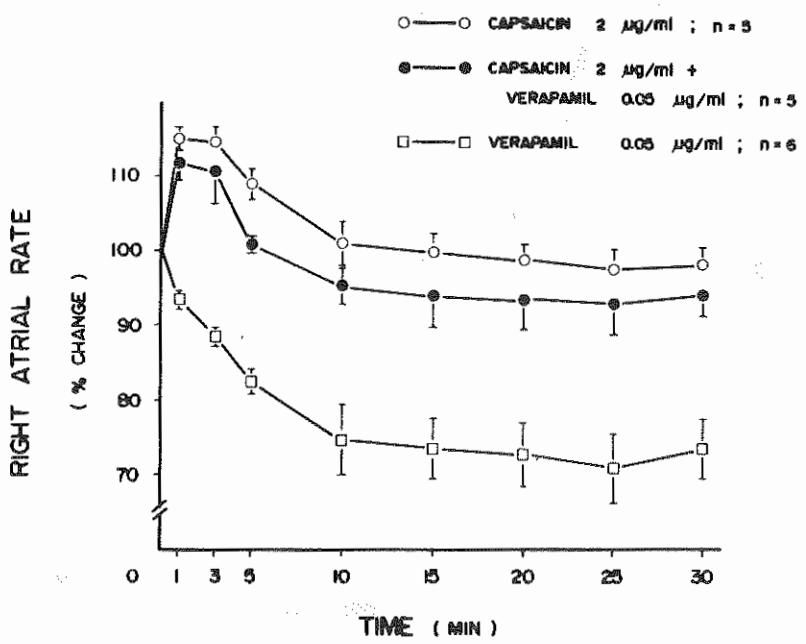

Figure 2. Antagonism of the negative chronotropic effect of verapamil by capsaicin. When both drugs were present, they were added simultaneously.

there was smaller reduction in isometric tension at 30 min of experimentation. The mean reduction in isometric force observed with verapamil (0.05 µg/ml) was about 47% at 30 min. Thus, verapamil was evidently more active on the left atrial force than on the right atrial rate. When both capsaicin and verapamil were present, the verapamil-induced negative inotropic response was clearly diminished while the acute stimulatory effect of capsaicin was insignificantly affected. Lower dose of capsaicin (2 µg/ml) was also found to significantly relieved, although to the less extent, the effect of verapamil on isometric tension. From Fig. 3, the 30 min isometric force increased from 52.95 ± 2.79% of controls with verapamil alone to 76.61 ± 5.02% when 10 µg/ml capsaicin was also present. The corresponding value when only 2 µg/ml capsaicin was added with verapamil was 64.96 ± 4.61%, n = 6 (results not included in Fig. 3). Likewise, from Fig. 2, the 30 min rates were raised from 73.39 ± 3.91% with verapamil alone to 94.29 ± 2.89% when 2 µg/ml capsaicin was co-administered. Thus, in term of the percent increase, capsaicin was apparently more effective in antagonizing verapamil effect on the rate than on the contractile force.

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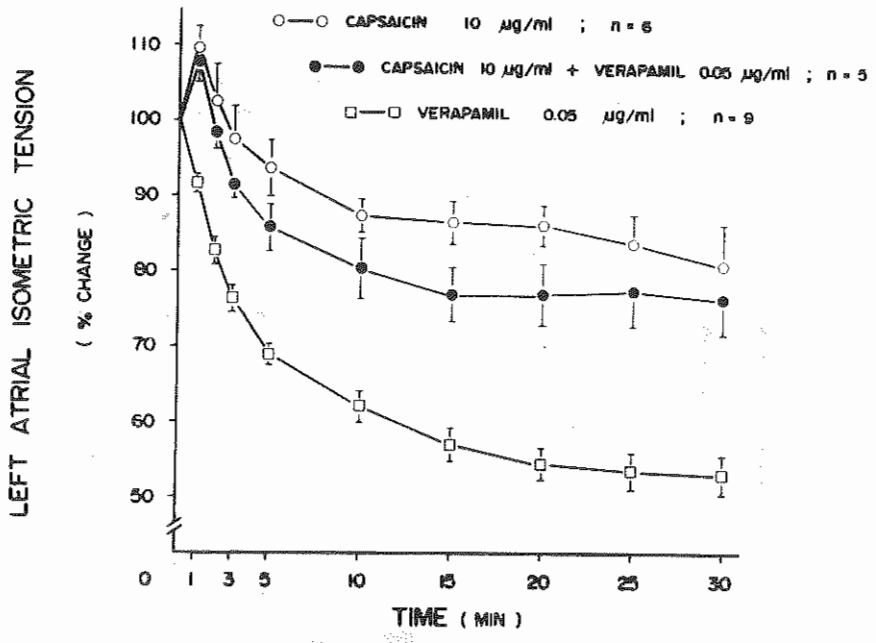

Figure 3. Antagonism of the negative inotropic effect of verapamil by capsaicin. When both drugs were present, they were added simultaneously.

Alleviation of the negative chronotropic and inotropic effect of verapamil by procainamide

The effects of procainamide (10 µg/ml), verapamil (0.05 µg/ml) and procainamide plus verapamil on the right atrial rate and left atrial isometric tension are depicted in Figs.4 and 5 respectively. When only procainamide was present, there was no significant stimulation of both the rate and contractile force. In fact, the drug appeared to cause slight atrial depression, particularly the isometric tension. When both drugs were added simultaneously, the depressive effect of verapamil was diminished. In both cases, the antagonism was significant from 15 to 30 min of experimentation. The 30 min rates increased from 73.39 ± 3.91% of controls with verapamil alone to 91.73 ± 4.50% when procainamide was also present; the corresponding values for the isometric force were the increase from 52.95 ± 2.79% to 70.03 ± 1.46%. Thus procainamide was about equally efficacious in attenuating varapamil effect on the rate and contractile force.

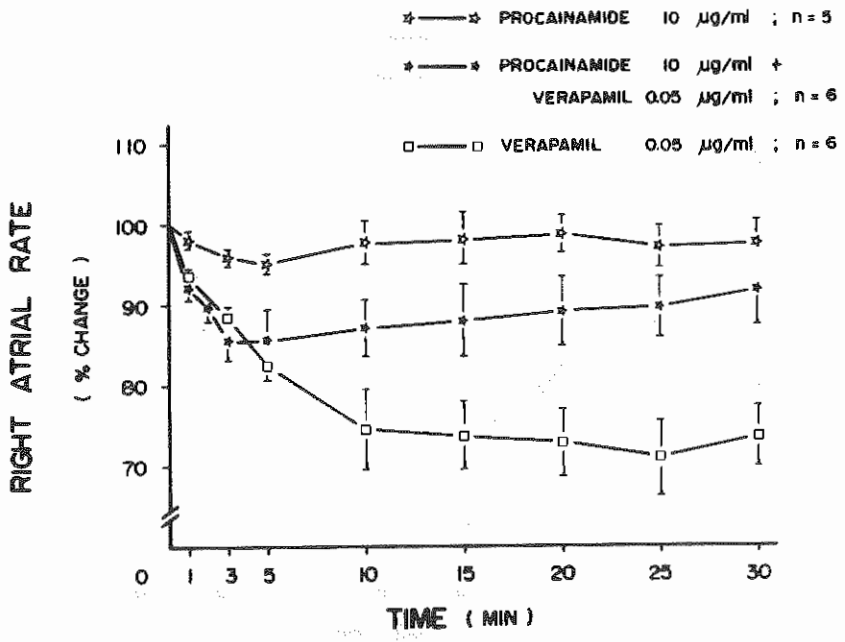

Figure 4. Attenuation of the negative chronotropic effect of verapamil by procainamide. Verapamil and procainamide were added concomitantly.

DISCUSSION

The results of the present study clearly show that capsaicin can elicit the positive chronotropic and inotropic response by isolated rat right and left atria respectively. Similar effect of capsaicin on isolated guinea pig atria has been reported previously (Molnar et al., 1969; Fukuda and Fujiwara, 1969). Whether this stimulation results from capsaicin acting directly on the atria is not clear at present. Recent evidence indicates that capsaicin can release and then deplete substance P from certain neurones (Nagy,1982; Buck and Burks, 1983). However, Kulakowski et al. (1983) found substance P to have no influence on aortic pressure, cardiac work and cardiac output in isolated rat heart. Reserpine pretreatment or prior addition of propranolol plus methysergide do not significantly alter the stimulant action of capsaicin on isolated rat atria (Naiwatanakul, 1984), suggesting that endogenous catecholamines and serotonin release is unlikely to participate. Whether capsaicin stimulates the atria by acting directly or indirectly, our results

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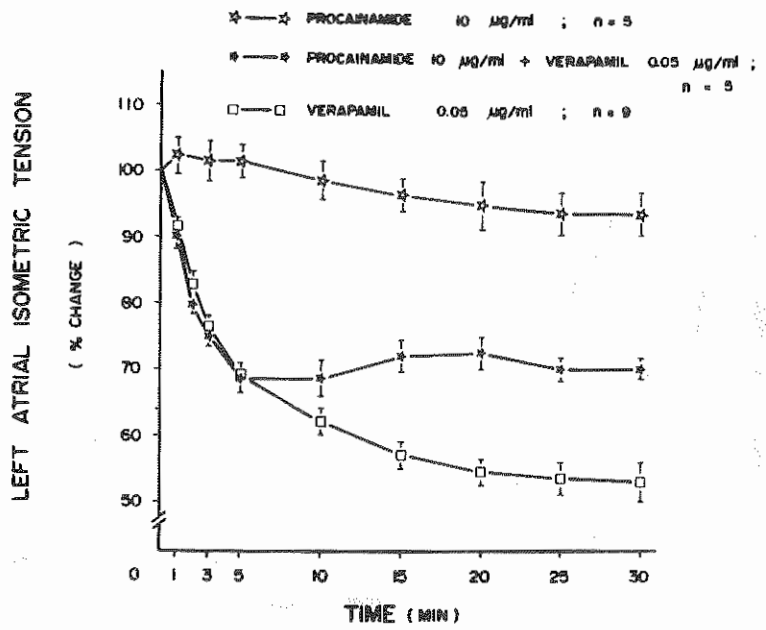

Figure 5. Attenuation of the negative inotropic effect of verapamil by procainamide. Verapamil and procainamide were added concomitantly.

show that verapamil, a drug which blocks slow inward calcium current (Fleckenstein, 1977) at the dose which prominently depress the atria has little or no effect on this process.

Perhaps the most interesting observation in this study is the alleviation of the verapamil-induced negative chronotropism and inotropism by capsaicin as well as procainamide. To our knowledge, this particular drug antagonism in isolated rat right and left atria has not been reported before. The possible mechanism of action is at best a matter of speculation. It is known that the rate and force depression produced by verapamil and other calcium antagonists is readily relieved by agents that increase transmembrane calcium supply, notably the β-adrenergic agonists (Fleckenstein, 1977). However, endogenous catecholamines release is unlikely to be the mechanism since reserpinization was found ineffective and procainamide is not known to release catecholamines. The capacity of procainamide to antagonize verapamil effect without exciting the atria suggests that the atrial stimulating activity of capsaicin may also not involve; although the two

compounds may possibly have different mechanism of actions. Thus both drugs, or at least procainamide,presumably act directly to attenuate verapamil effect on isolated rat atria. Structural inspection of verapamil, capsaicin and procainamide reveals common feature, i.e., their molecules composed of aromatic ring connected to the nitrogen-containing lipophilic side chain. We, therefore, hypothesize that capsaicin and procainamide may interfere with the binding of verapamil on sarcolemmal binding site (Cohen et al., 1984). Certainly much more work is needed to determine the exact mechanism of this drug interaction as well as its pharmacological and/or toxicological significance.

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