# CHAPTER III

## RESULTS

### A. Depressive action of capsaicin on the spontaneous rate of isolated rat atria

Illustrative tracings showing heart rate depression by capsaicin are depicted in Figure 2. Capsaicin, at 20 µg/ml, was found to progressively reduce the spontaneous rate of isolated rat atria. In most experiments, this negative chronotropic effect can be detected as early as 1 min after capsaicin. It had also been observed in some experiments however, that capsaicin produced an initial increase followed by a decrease in atrial rate. The atrial-slowing action of capsaicin invariably terminated with complete cessation of heartbeat. Irregular beats conspicuously occurred in many experiments before the atria were quiescent. Generally, the atria stopped beating when there was opproximately a 35-50 % reduction in the rate. Figure 3 shows the titration curves of capsaicin effect on atrial rate. Control atria receiving only equal volume of vehicle (ethanol) exhibited minute decrease in the rate during the 30 min period. As would be expected, the negative chronotropic effect was more prominent with high dose of capsaicin. At 10 µg/ml capsaicin, 7 of 15 atria ceased to beat within 30 min after capsaicin was added. Increasing the dose to 20 µg/ml caused atrial rate to fall more rapidly and 8 of 12 atria failed to beat within 15 min after capsaicin addition. The time from capsaicin addition to complete stop of heartbeat were $35.5 \pm 4.7$ min (ranging from 16 to 72 min; n = 15) and $12.1 \pm 1.1$ min (ranging from 5 to 18 min. n = 12) for

10 and 20 µg/ml capsaicin respectively. It is noteworthy that 8 of 15 atria responded to low dose of capsaicin (10 µg/ml) with the initial increase in rate whereas only 3 of 12 atria responded in the same manner with higher dose (20 µg/ml).

The effect of capsaicin on atrial rate was reversible as shown in Figure 4. In these experiments, 20 µg/ml capsaicin was first added to induce cessation of heartbeat. Three minutes after the atrial rate had ceased, the atria were then washed ten times each with 25-ml portion of capsaicin-free medium. This procedure was found to restore regular beat and almost normal rate. Interestingly, these washed atria were ostensibly more responsive to another addition of capsaicin (20 µg/ml) since the decline in atrial rate and termination of heartbeat occurred more abruptly than those found with the atria not receiving the chemical beforehand.

In order to determine whether the negative chronotropic effect of capsaicin involved acetylcholine release from parasympathetic nerve endings in the atria, experiments were performed with atropine present. As reported in Table I, prior addition of atropine at concentration as high as 0.24 µg/ml did not mitigate capsaicin effect on atrial rate. Capsaicin still retained its negative chronotropic and arrhythmogenic action in the presence of atropine at sufficiently high concentration to block acetylcholine action on muscarinic cholinergic receptor. Similarly, raising the concentration of calcium chloride in the medium offered no protection against capsaicin. Calcium chloride, at 43 µmoles, added before (Table II) or after (Table III) capsaicin (20 µg/ml) did not alleviate the deleterious action of capsaicin on the atria. In one experiment, addition of

215 μmoles calcium chloride tothe capsaicin-induced, non-beating atrium was found ineffective to initiate heartbeat.

B. Antagonism of capsaicin effect on atrial rate by catecholamines

It is well known that catecholamines possessing $\beta_1$-stimulating activity, for instance isoproterenol, have direct positive chronotropic and inotropic effect on the heart. Since capsaicin was found to depress atrial rate, it was therefore interesting to investigate whether this compound could modify the positive chronotropic effect of catecholamines on isolated atria, and vice versa. As resported in Table IV, isoproterenol (0.08 μg/ml) added to the atria produced dramatic increase in atrial rate. This increase was sustained during the 5 min period of experimentation. When isoproterenol was added 5 min following capsaicin (20 μg/ml), the impairment of the positive chronotropic effect was evident. Most interestingly, however, was the observation that 2 of 3 atria continued to beat for much longer period than would be expected if only capsaicin was present. This finding indicated the antagonism of the capsaicin-induced heart rate depression by isoproterenol. This conclusion was confirmed by other experiments in which isoproterenol was added after the capsaicin treated atria had stopped beating (Table V). In this study, capsaicin (10 μg/ml) was first added to induce quiescent atria. Addition of isoproterenol (0.08 μg/ml) 3 min after termination of atrial rate not only restored regular beat but also stimulated the atria to beat at higher rate than control. The capsaicin-treated atria continued beating at the rate higher than control even 60 min after isoproterenol was added. No arrhythmias was observed following isoproterenol addition.

Experimental results comparing the effects of the three $\beta_1$-stimulating catecholamines, namely isoproterenol, epinephrine, and norepinephrine, on the non-beating atria produced by capsaicin (10 µg/ml) were recorded in Figure 5. It is seen that isoproterenol and norepinephrine was the most and least efficacious catecholamines in restoring atrial rate respectively. Thirty minutes after the addition of catecholamines (0.08 µg/ml), isoproterenol restored atrial rate to value above control while norepinephrine caused the atria to resume beating at the rate much slower than control. The effect of epinephrine was evidently less intense than isoproterenol but stronger than norepinephrine. However, the ability of the less potent catecholamines to antagonize capsaicin action on atrial rate can be enhanced by raising the amines concentrations as shown in Table VI. In these experiments the antagonistic effects of low (0.08 µg/ml) and high (0.4 µg/ml) concentrations of epinephrine were compared. Although both high and low doses of epinephrine were capable of initiating atrial beat when added to the capsaicin-induced, dormant atria, high dose stimulated the atria to beat at sustained faster rate than with low concentration.

Figures 6 and 7 demonstrated that the heartbeat-restoring effect of the three catecholamines was obliterated by propranolol, a β-adrenergic receptor blocking agent. In experiments reported in Figure 6, propranolol (0.8 µg/ml) added shortly after termination of heartbeat caused by capsaicin (20 µg/ml) completely abolished the heartbeat-initiative activity of all three amines each given in two consecutive does (0.32 µg/ml total doeses) at 3 and 8 min after propranolol. Likewise, propranolol (0.2 µg/ml) added 30 min after isoproterenol (0.16 µg/ml) reduced the rate followed by cessation of the isoproterenol-

mediated heartbeat (Figure 7). The relatively slow blocking action of propranolol when added after isoproterenol presumably due to the small concentration employed in this study.

C. Effect of electrical stimulation on the non-beating atria induced by capsaicin

The results described above clearly demonstrated the depressive action of capsaicin on atrial rate which was reversed by $\beta_1$-stimulating catecholamines. This depressive effect consistently ended with heartbeat termination. There are, at least, two possiblity which must be considered regarding how capsaicin mediated the heartbeat cessation. Firstly, capasaicin may act on SA node to prevent impulse generation and/or conduction. Secondly, capsaicin may interfere with the atrial excitation-contraction coupling mechanism and rendered the atria unresponsive to electrical impulse from SA node. In order to test these hypotheses, the effect of electrical stimulation on the capsaicin induced, non-beating atria was studied. As reported in Figure 8, electric current applied to the capsaicin-treated, quiescent atria elicited regular contraction. Moreover, the atria can be electrically driven to beat at the rate as high as 300/min with no evidence of arrhythmias. Note also the decrease in contractile force with increasing stimulus frequency. These experiments ruled out the impairment of excitation-contraction coupling mechanism from being responsible for the heartbeat cessation caused by capsaicin.

D. Depression by capsaicin of isometric force by electrically paced left atria

All the experiments carried out up to this point had been

performed with whole atria, i.e., having both right and left sides. With these atrial preparations, capsaicin was found to produce variable effect on the contractile force. The most probable reason behind this is that the contractile force of isolated rat atria varies inversely, at least to some extent, with the rate (see also Figure 8). The force tends to rise as the rate falls and to decline as the rate increases. Thus, to study capsaicin effect on atrial force, it is necessary to maintain constant rate. This is achieved by using left atria electrically stimulated at constant frequency. Figure 9 shows capsaicin effect on isometric tension by left atria electrically paced to beat at constant rate of 250/min. Capsaicin (20 µg/ml) caused about 40 % diminution in isometric force 30 min after the chemical was added. Although the force appeared to increase at 1 min, this did not differ significantly from control receiving only the vehicle. The depressed contractile force induced by capsaicin can be augmented by adding norepinephrine or calcium chloride (Figure 10).

E. Effect of methyl capsaicin on the spontaneous rate of isolated rat atria

This effect of methyl capsaicin, the non-phenolic derivative, on atrial rate is recorded in Figure 11. Like capsaicin, methyl capsaicin at 20 µg/ml caused a gradual reduction in atrial rate which terminated with cessation of heartbeat. After repeated washings the beating resumed. However, contrary to capsaicin, methyl capsaicin seemed less effective than the parent compound in terminating the heartbeat since 9 of 13 atria continued to beat for more than 15 min after methyl capsaicin was added. In addition, the force of the washed atria remained much depressed compared to control.

Figure 12 shows that isoproterenol can effectively antogonize the action of methyl capsaicin on atrial rate. Isoproterenol (0.08 µg/ml) added to the quiescent atria induced by methyl capsaicin (20 µg/ml) restored regular beat. The atrial rate after isoproterenol was sustained close to the control during the 60 min period of experimentation.

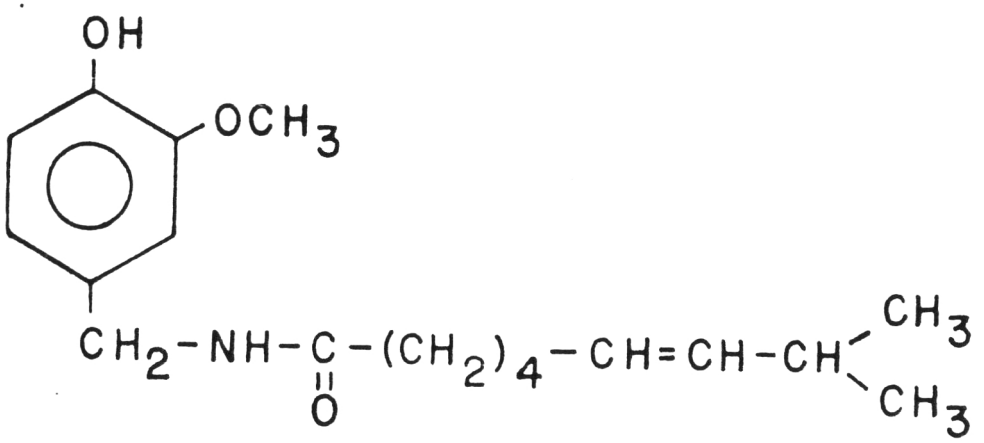


Figure 1. Chemical structure of capsaicin

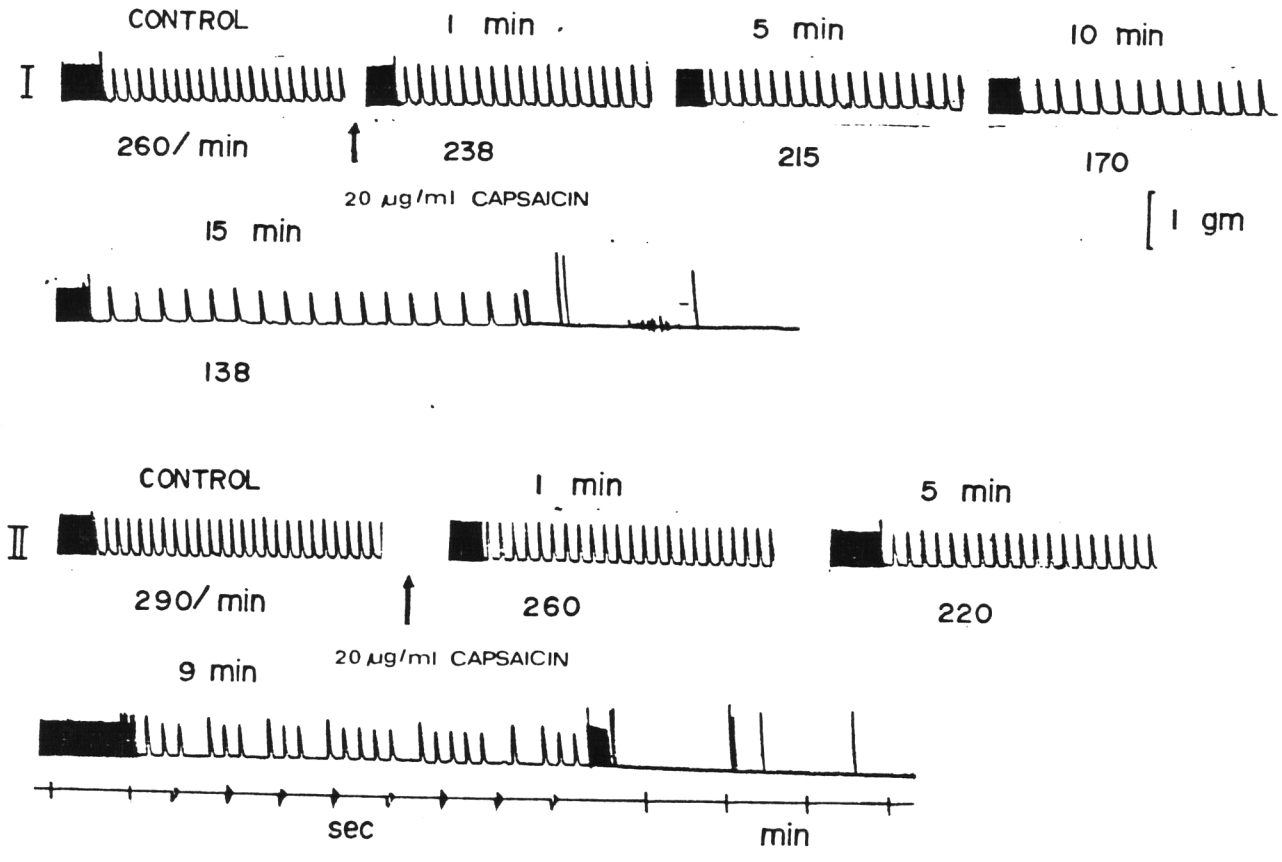


Figure 2. Negative chronotropic and arrhythmogenic effects of capsaicin on isolated rat atria

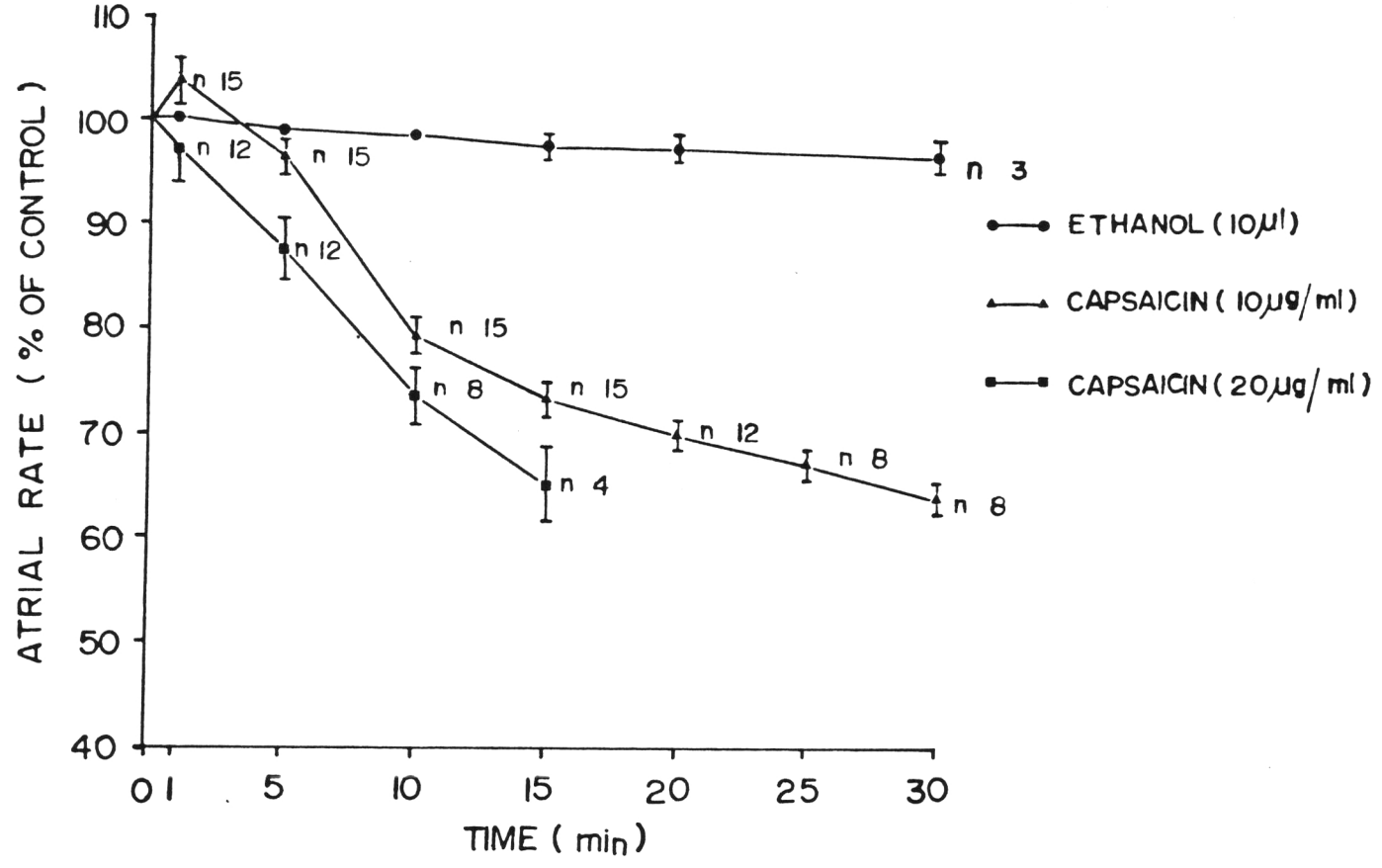


Figure 3. Dose-response curve of the depressive effect of capsaicin on atrial rate

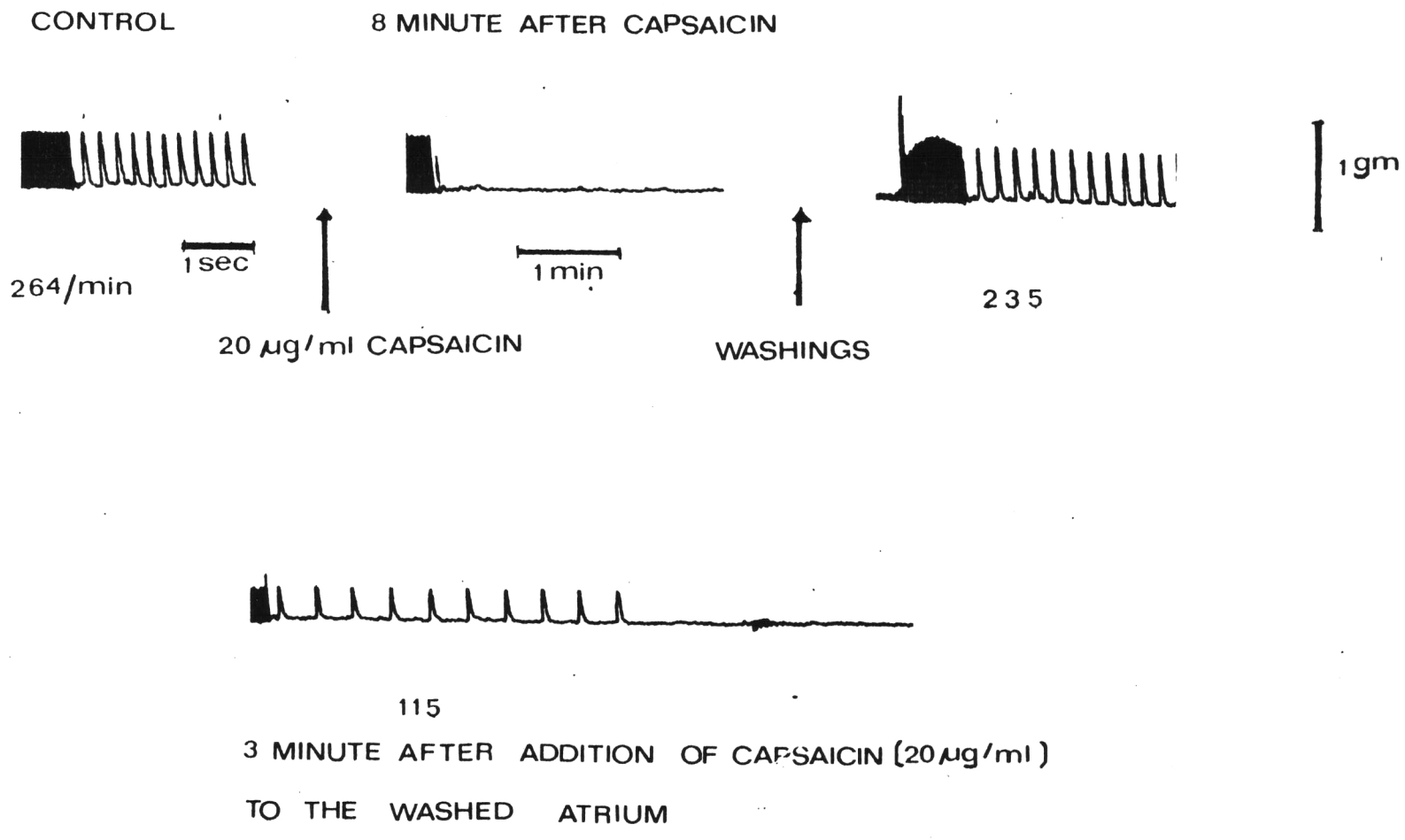


Figure 4. Restoration of heartbeat by repeated washings of the non-beating atria produced by capsaicin

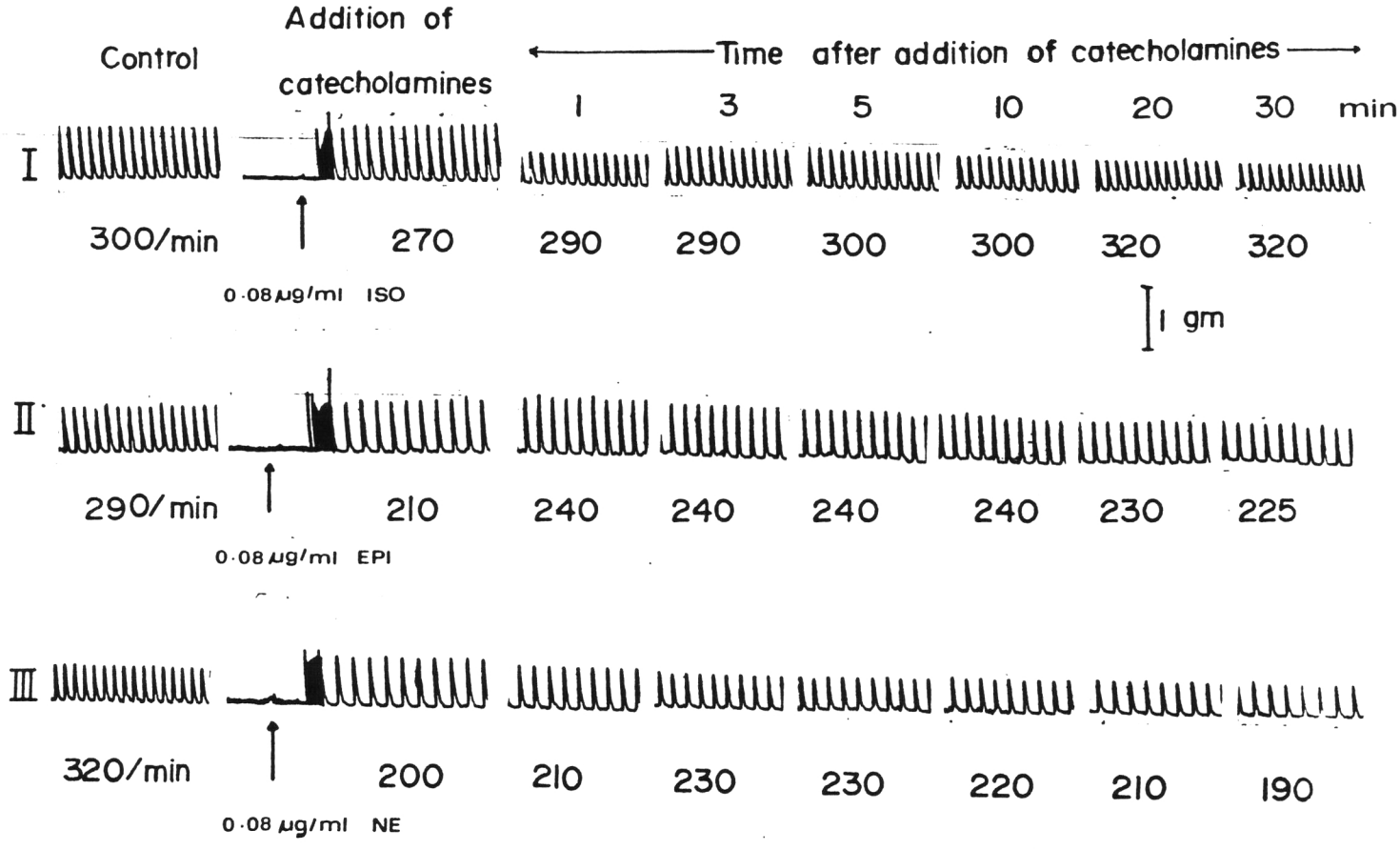


Figure 5. Reversal of the capsaicin-induced cessation of heartbeat by catecholamines

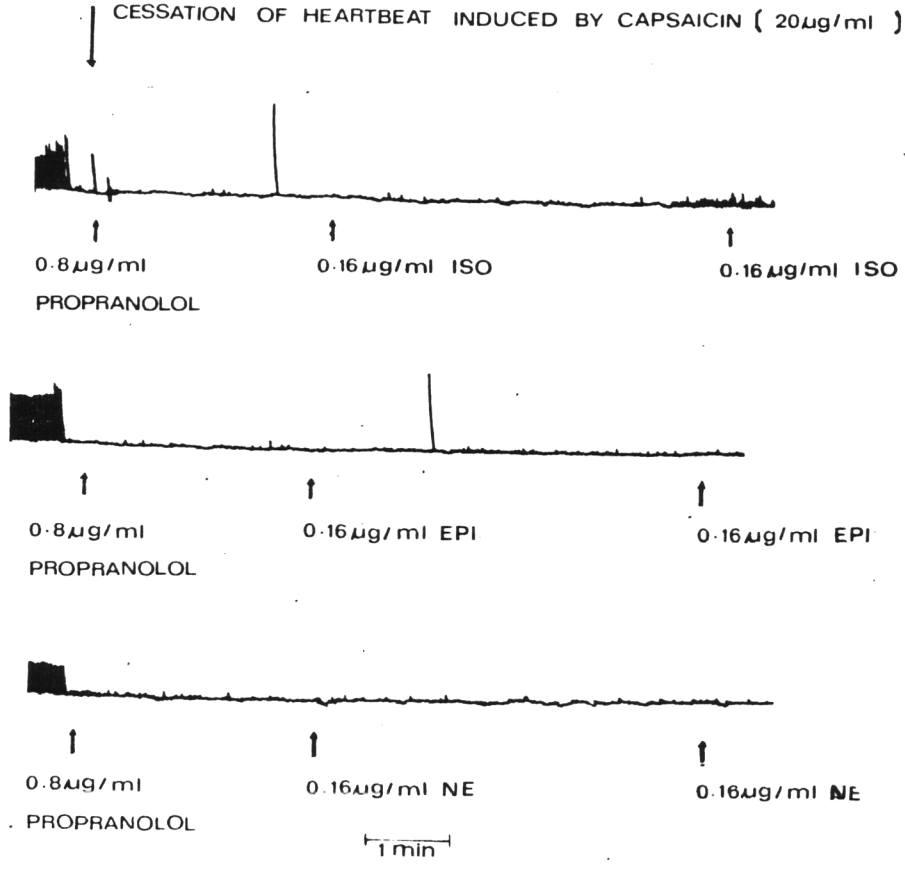


Figure 6. Blockade of the heartbeat-initiative effect of catecholamines by prior addition of propranolol

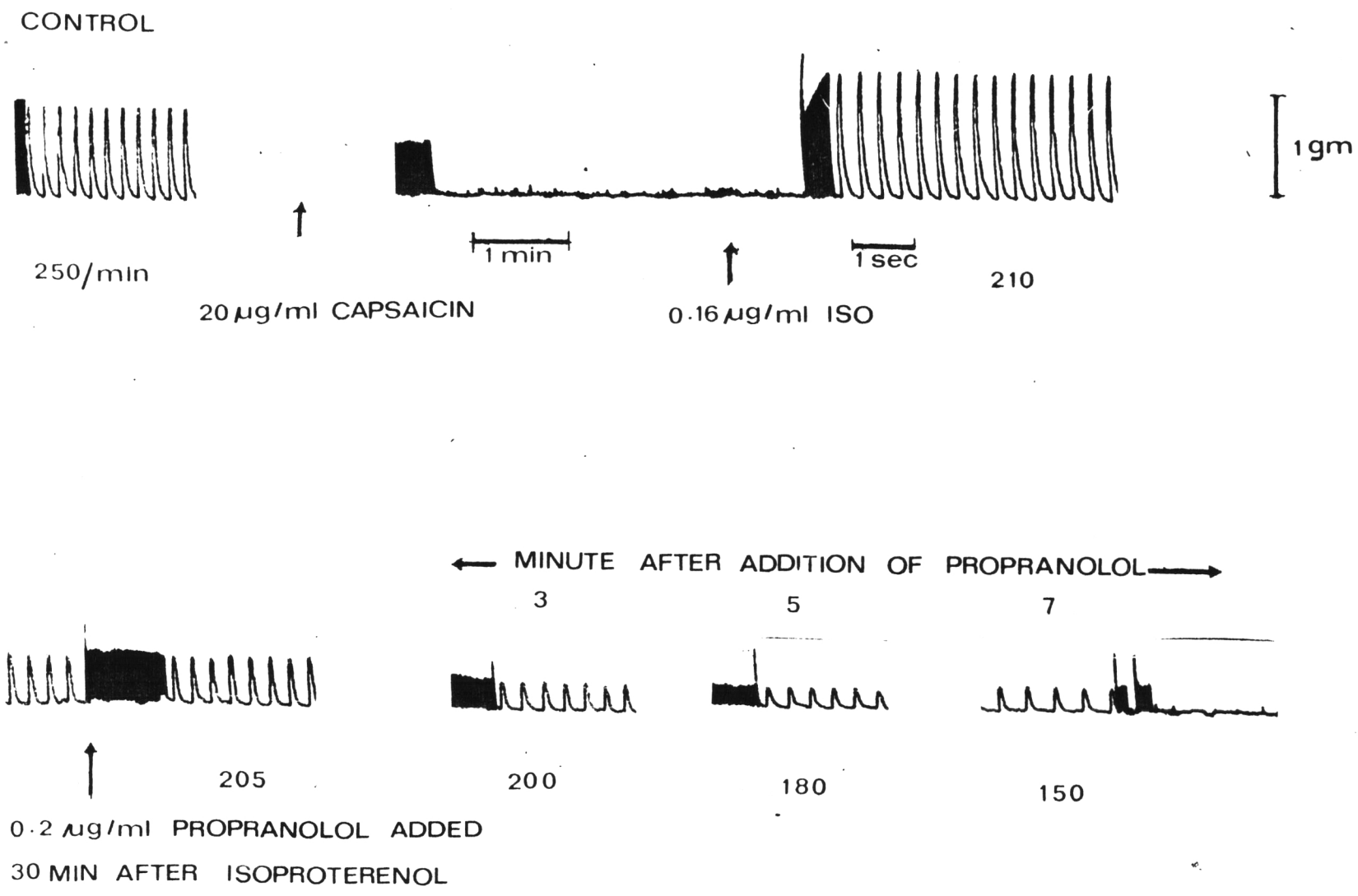


Figure 7. Blockade of the heartbeat-initiative effect of isoproterenol by subsequent administration of propranolol

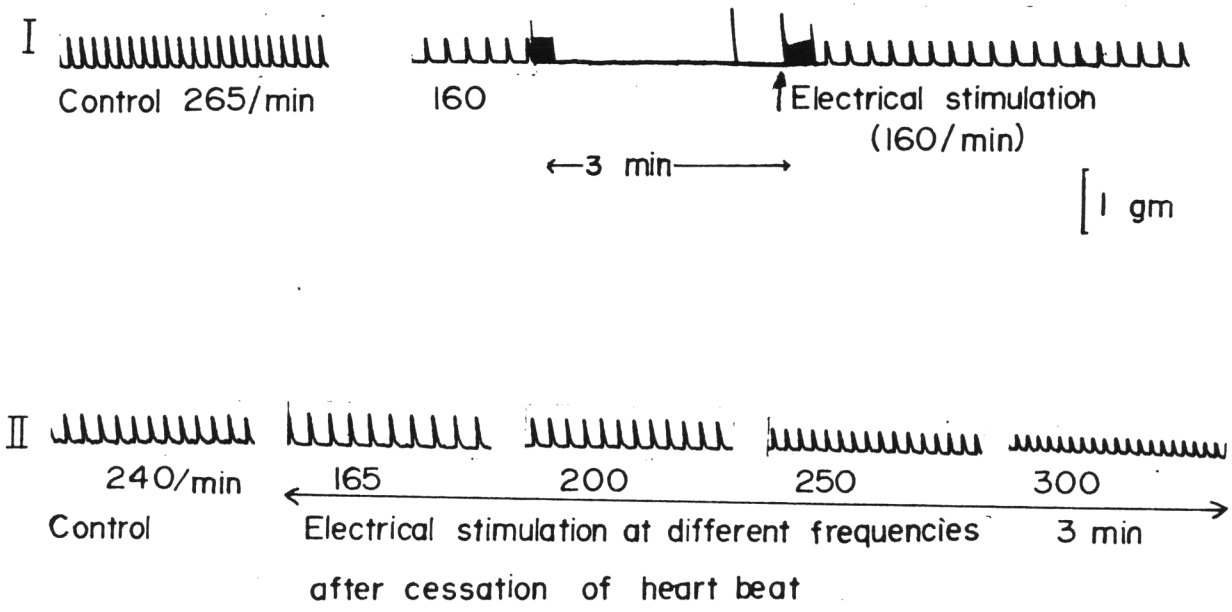


Figure 8. Effect of electrical stimulation on the non-beating atria induced by capsaicin

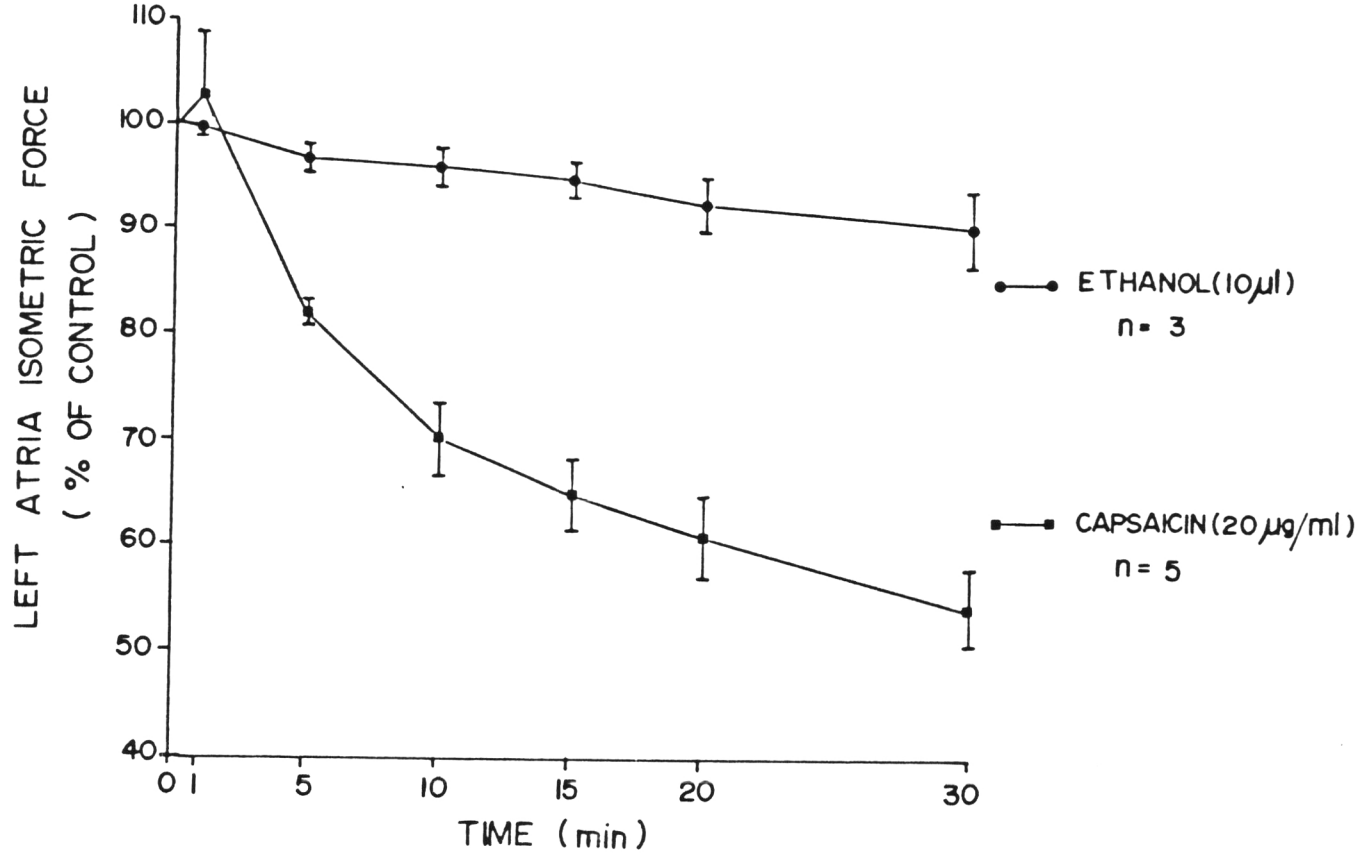


Figure 9. Dose-response curve of the depressive effect of capsaicin on isometric tension of electrically driven left atria

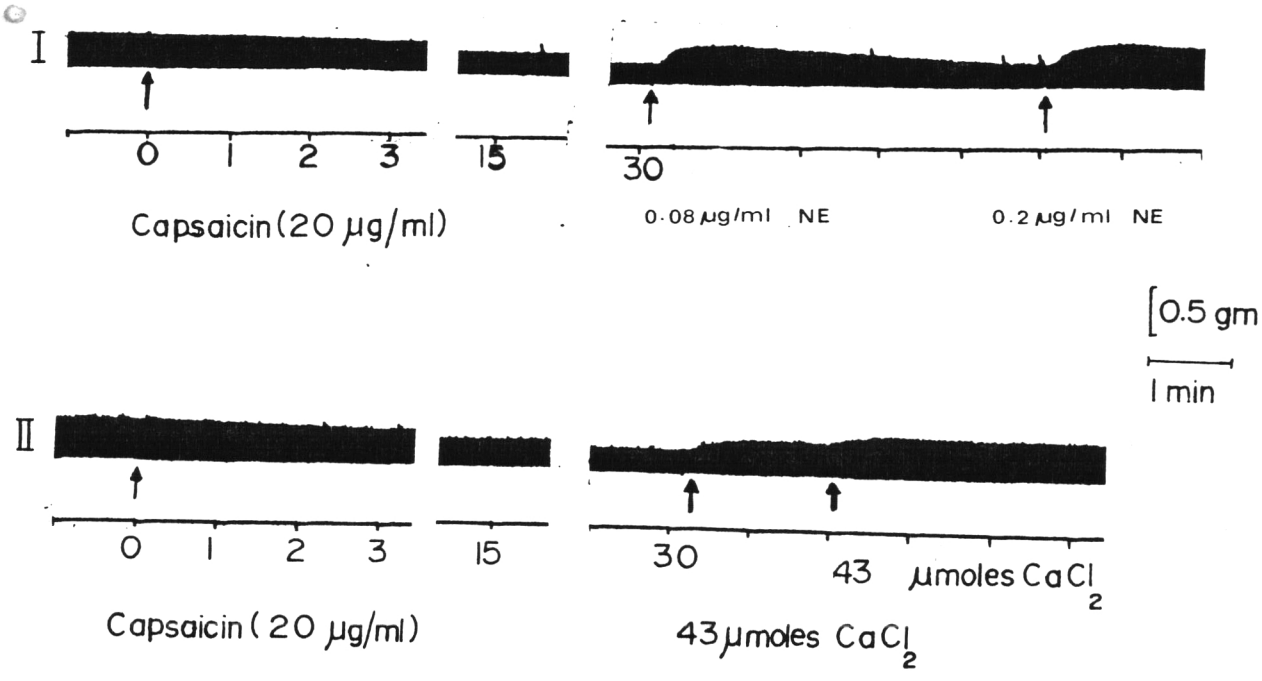


Figure 10. Augmentation of isometric force of capsaicin-treated left atria by norepinephrine and calcium chloride

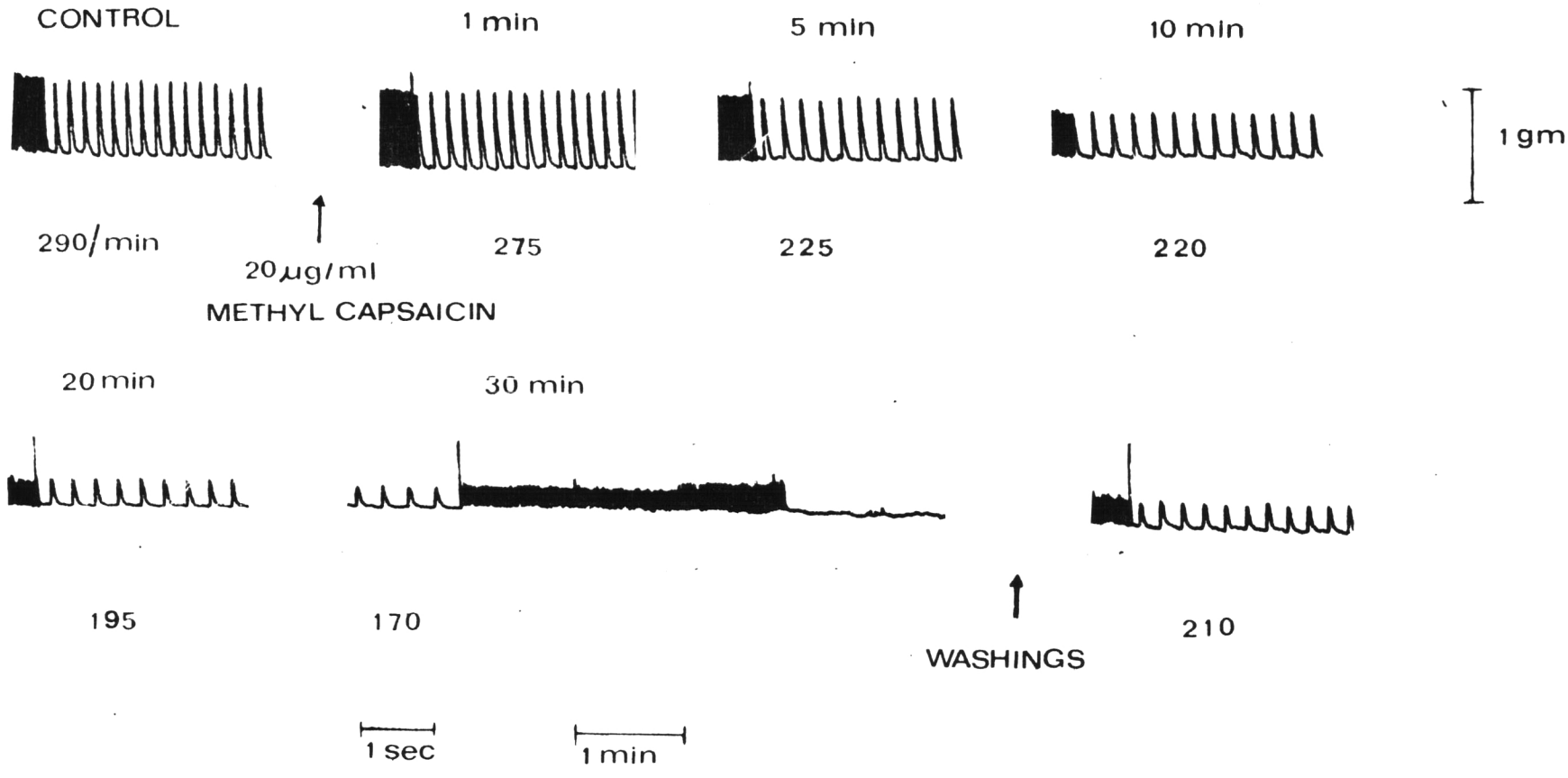


Figure 11. Depressive effect of methyl capsaicin on atrial rate and restoration of heartbeat by repeated washings

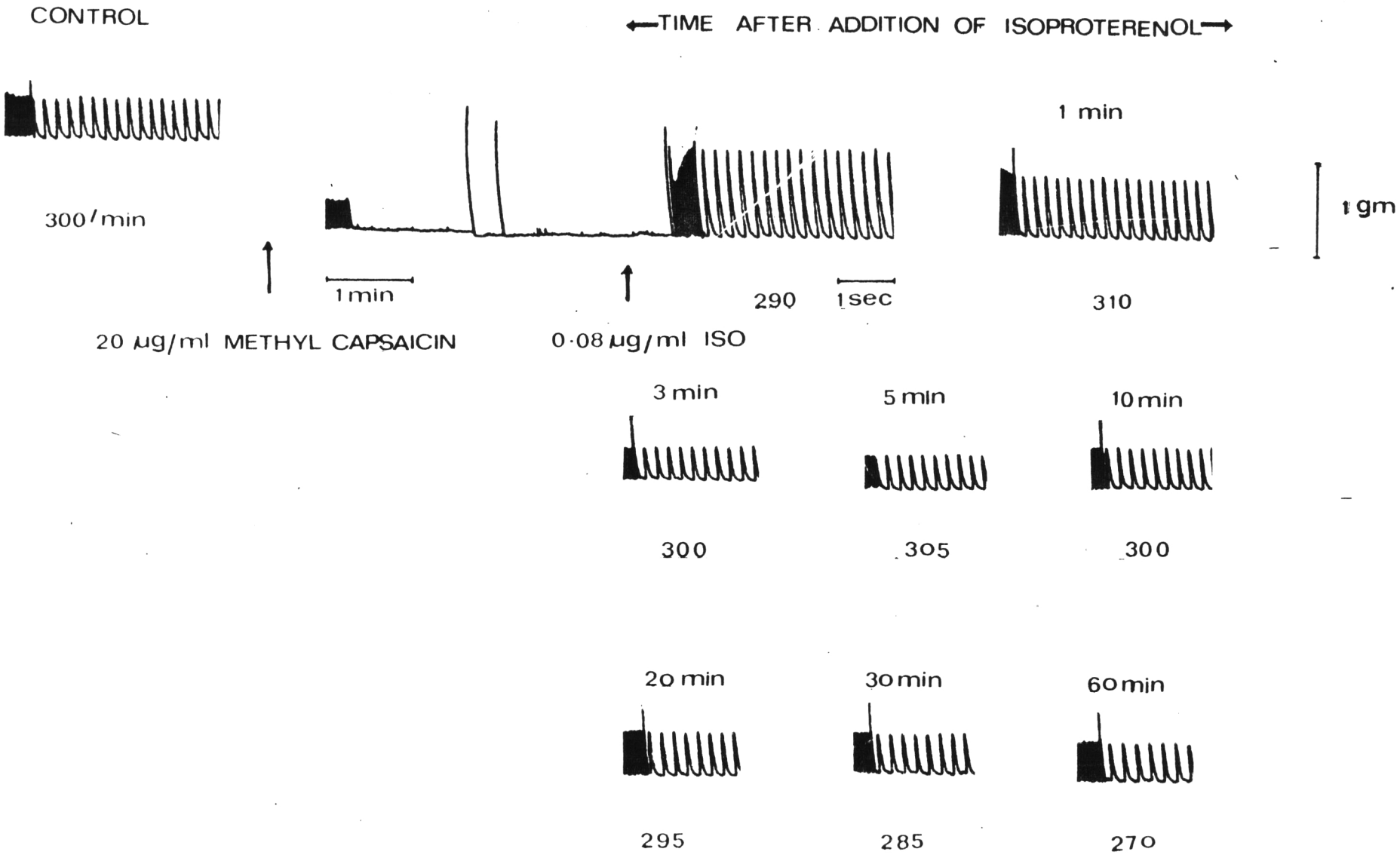


Figure 12. Reversal of the methyl capsaicin-mediated cessation of heartbeat by isoproterenol

Table 1

Effect of atropine on the capsaicin-induced depression of atrial rate

| Experiment number | atrial rate (beat/min) | | | | | Cessation of heartbeat (min after capsaicin) | Remarks |
|---|---|---|---|---|---|---|---|
| | control | 1 min after atropine | min after addition of capsaicin | | | | |
| | | | 1 | 5 | 10 | | |
| 1 | 250 | 255 | 230 | 175 | — | 7 | |
| 2 | 250 | 250 | 235 | 220 | 180 | 11 | arrhythmias started 10 min after capsaicin |
| 3 | 285 | 275 | 270 | 225 | 180 | 15 | arrhythmias started 11 min after capsaicin |

The concentrations of atropine were 0.12 µg/ml in experiment no. 1 and 0.24 µg/ml in experiment no. 2 and 3.

The concentration of capsaicin was 20 µg/ml in all experiments. Capsaicin was added 1 min after atropine.

Table II

Effect of calcium chloride added prior to capsaicin on the capsaicin-induced depression of atrial rate

| Experiment number | Atrial rate (beat/min) | | | | | | | Cessation of heartbeat (min after capsaicin addition) | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | Control | 1 min after $CaCl_2$ addition | min after capsaicin addition | | | | | | |
| | | | 1 | 5 | 10 | 15 | 20 | | |
| 1 | 285 | 300 | 250 | | | | | 5 | arrhythmias started 4.5 min after capsaicin |
| 2 | 285 | 290 | 240 | 210 | 170 | 155 | 148 | 21 | |
| 3 | 260 | 260 | 250 | 195 | 155 | | | 11 | |
| 4 | 320 | 330 | 320 | 220 | | | | 11 | arrhythmias started 8 min after capsaicin |

The concentrations of capsaicin and calcium chloride were 20 µg/ml and 43 µmoles respectively. Capsaicin was added 2 min after calcium chloride.

Table III

Effect of calcium chloride added after capsaicin on the capsaicin-induced depression of atrial rate

| Experiment number | Atrial rate (beat/min) | | | | | | Cessation of heartbeat (min after $CaCl_2$ addition) | Remarks |
|---|---|---|---|---|---|---|---|---|
| | Control | 1.5 min after capsaicin addition | min after $CaCl_2$ addition | | | | | |
| | | | 1 | 5 | 10 | 15 | | |
| 1 | 270 | 225 | 170 | | | | 7 | arrhythmias started 4 min after $CaCl_2$ |
| 2 | 260 | 220 | 205 | 180 | 170 | 95 | 18 | arrhythmias started 17 min after $CaCl_2$ |
| 3 | 270 | 210 | 190 | 150 | 120 | | 13 | arrhythmias started 12 min after $CaCl_2$ |
| 4 | 250 | 220 | 190 | | | | 4 | arrhythmias started 3 min after $CaCl_2$ |
| 5 | 270 | 225 | 210 | 180 | 145 | | 11 | |

The concentrations of capsaicin and calcium chloride were 20 µg/ml and 43 µmoles respectively. Calcium chloride was added 2 min after capaicin.

Table IV

Interference by capsaicin of the positive chronotropic effect of isoproterenol on isolated rat atria

| Experiment number | Percent increase in atrial rate | | | | | | Cessation of heartbeat (min after capsaicin) |
|---|---|---|---|---|---|---|---|
| | 0.08 µg/ml isoproterenol | | | 20 µg/ml capsaicin + 0.08 µg/ml rate isoproternal | | | |
| | 0.5 min | 1 min | 5 min | 0.5 min | 1 min | 5 min | |
| 1 | 44 | 55 | 61 | 10 | 20 | 15 | 47 |
| 2 | 58 | 73 | 66 | 36 | 61 | 57 | 72 |
| 3 | 30 | 30 | 33 | 39 | 43 | 21 | 15 |

Percent changes in atrial rate were measured following the addition of isoproterenol. When both capsaicin and isoproterenol were present, isoproterenol was added 5 min after capaicin.

Table V

Reversal by isoproterenol of the capsaicin-induced cessation of heartbeat

| Experiment numbers | Atrial rate (beat/min) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | min after addition of isoproterenol to the non-beating atria caused by capsaicin | | | | | | | | | |
| | | 0.5 | 1 | 3 | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 |
| 1 | 300 | 270 | 290 | 290 | 300 | 300 | 310 | 320 | 320 | 320 | 325 | 330 |
| 2 | 290 | 300 | 300 | 310 | 320 | 320 | 330 | 340 | 350 | 340 | 340 | 330 |
| 3 | 260 | 300 | 280 | 270 | 280 | 280 | 280 | 280 | 290 | 290 | 300 | 300 |
| 4 | 260 | 280 | 290 | 290 | 290 | 280 | 280 | 280 | 280 | 290 | 290 | 290 |

The concentrations of capsaicin and isoproterenol were 10 and 0.08 μg/ml respectively. Isoproterenol was added 3 min after the capsaicin-treated atria stopped beating.

Table VI

Antagonism of the capsaicin-induced cessation of heartbeat by low and high doses of epinephrine

| Experiment number | Concentration of epinephrine (μg/ml) | Atrial rate (beat/min) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | min after addition of epinephrine to the non-beating atria caused by capsaicin | | | | | | | | | |
| | | | 0.5 | 1 | 3 | 5 | 10 | 15 | 20 | 30 | 40 | 50 | 60 |
| 1 | 0.08 | 290 | 210 | 240 | 240 | 240 | 240 | 230 | 230 | 225 | 220 | 200 | 200 |
| 2 | 0.08 | 320 | 160 | 160 | 210 | 230 | 165 | 170 | 160 | 160 | 160 | 150 | arrhythmias |
| 3 | 0.4 | 300 | 250 | 260 | 255 | 265 | 270 | - | 270 | 270 | 270 | 250 | 270 |
| 4 | 0.4 | 330 | 290 | 295 | 300 | 305 | 310 | - | 300 | 300 | 300 | 295 | 290 |
| 5 | 0.4 | 300 | 260 | 270 | 260 | 270 | 275 | - | 280 | 290 | 290 | 285 | 285 |

The concentration of capsaicin was 10 μg/ml. Epinephrine was added 3 min after the beating of the capsaicin-treated atria terminated.