LETTER TO THE EDITOR:

INTERACTION OF ACETYLCHOLINE AND CAFFEINE ON THE ISOLATED RECTUS ABDOMINIS OF FROG (RANA TIGRINA)

Sir,

During the study on the effect of essential oil of Psoralia corylifolia on isolated skeletal muscles (6), it was observed that acetylcholine reduced the responses induced by caffeine in the isolated rectus abdominis of frog. This effect of acetylcholine has been analysed in more details in the present study.

Isolated rectus abdominis of frog was mounted in aerated frog Ringer solution (4) in a 25 ml bath at 10°C since the sensitivity of this tissue is increased at 10°C (2). The contractions were recorded with Gimbal lever giving a 12-fold magnification. Contractions were induced by either caffeine (4x10^-3M) or potassium chloride (1x10^-2M) and the effect of subthreshold concentrations of acetylcholine bromide (4.4x10^-9M) was studied on these responses by incubating the preparation with acetylcholine for 5 min prior to the addition of caffeine or potassium. The effect of procaine hydrochloride (2x10^-4M) and manganese chloride (5x10^-3M) on the responses to caffeine and potassium was also observed. The influence of manganese, on the effect of acetylcholine on caffeine-induced contractures, was also recorded.

It was found that acetylcholine caused a significant increase in the latent period of contraction induced by caffeine. The latent period increased from 60 ± 5 sec to 150 ± 12 sec (n=12, P>0.001). It also inhibited the response of the muscle to caffeine, the degree of inhibition being proportional to the concentration of acetylcholine added (Table I). The same concentration of acetylcholine, however, increased the responses to caffeine-induced contractures, was also recorded.

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<tr>
<th>Concentration of acetylcholine</th>
<th>Percentage inhibition* mean ± SE.</th>
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<td>2.2 x 10^-9 M</td>
<td>11.34 ± 1.6</td>
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<tr>
<td>4.4 x 10^-9 M</td>
<td>21.66 ± 2.1</td>
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<tr>
<td>8.8 x 10^-9 M</td>
<td>27.5 ± 2.2</td>
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<tr>
<td>1.76 x 10^-8 M</td>
<td>35.00 ± 2.5</td>
</tr>
<tr>
<td>3.52 x 10^-8 M</td>
<td>56.00 ± 1.7</td>
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* n = 12
of the muscle to potassium (Fig. 1 — panel I, n=5). Procaine and manganese on the other hand, reduced the responses of the muscle to both caffeine and potassium (Fig. 1 — panels II & III, n=4). In the presence of manganese, the inhibitory effect of acetylcholine on caffeine contractures was not seen (Fig. 1 — panel IV, n=5).

![Diagram](image)

**Fig. 1:** Isolated rectus abdominis of frog (Rana tigrina). (M = 0.1 M saline) + acetylcholine to panel I. 

Panel I — Effect of acetylcholine (4.4 x 10^-9 M, at A) on caffeine (4 x 10^-3 M, at C) and potassium (1 x 10^-2 M, at K) induced contractions.

Panel II — Effect of procaine (2 x 10^-4 M, at P) on potassium (1 x 10^-2 M, at K) and caffeine (4 x 10^-3 M, at C) induced contractions.

Panel III — Effect of manganese (5 x 10^-3 M, at M) on potassium (1 x 10^-2 M, at K) and caffeine (4 x 10^-3 M, at C) induced contractions.

Panel IV — Effect of acetylcholine (4.4 x 10^-9 M, at A) and manganese (5 x 10^-3 M) + acetylcholine (at MA) on caffeine (4 x 10^-3 M, at C) induced contractures.

Acetylcholine, procaine and manganese were allowed to act for 5 minute. The effect of caffeine was recorded for 5 min. while that of potassium was recorded for 2 minutes.

Caffeine induces contraction of the skeletal muscle by labilisation of calcium induced calcium release (1) and by translocation of calcium from the sarcoplasmic reticulum.
culum (5). The effect of caffeine on the skeletal muscle also depends on the membrane potential and it is suggested that a part of the stimulant effect of caffeine is mediated through the 'T' tubules (2). It appears from the above observations, that the inhibitory effect of acetylcholine on caffeine contractures is probably due to the blockade of that part of action of caffeine which is mediated through the 'T' tubules. This is because there is a significant increase in the latent period of contraction after acetylcholine and there is an antagonism between acetylcholine and manganese, when added together. This action of acetylcholine, however, appears to be different from that of manganese, which blocks the transfer of impulse from the 'T' tubules to the lateral sacs (3), since the former only inhibits the action of caffeine while the latter inhibits the effect of both caffeine and potassium.

S.S. RAO, A. W. BHAGWAT AND V. G. PARMANAND

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REFERENCES