A Dual Effect of Acetylcholine on Gastropod Smooth Muscle Preparations

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Pharmacological studies on gastropod smooth muscle preparations have led us to the conclusion that the muscles *in vitro* behaves as if supplied by both excitatory and inhibitory cholinergic receptor sites.

At the neuromuscular junction of certain molluscan smooth muscles acetylcholine (ACh) is thought to be released by motoneuron activity eliciting muscle contraction [1]. The discovery, however, that isolated gastropod smooth muscle preparations are not very sensitive to exogenously applied ACh [2, 3] and that constant maximum contraction amplitudes were only obtained after 4 or 5 successive administrations of ACh (Fig. 1 a) should not be ignored. In this connection it is important to remember that the action of ACh may be artifactual in the sense that the initial purely excitatory effect

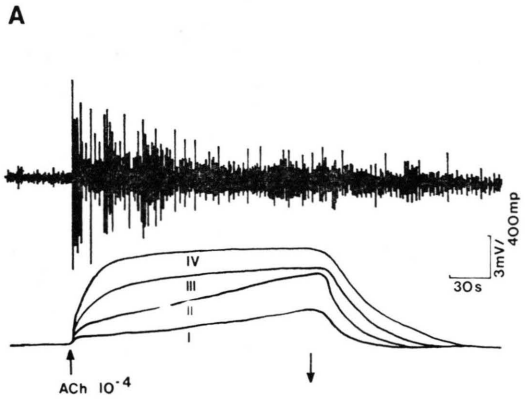

Fig. 1. a. Effect of repeated administrations of ACh on the PRM. The muscle was perfused with Ringer's solution [6] in which ACh was injected to a final concentration of 10^{-4} M and was thoroughly washed out again after a 3 min incubation period. After washing periods of ten minutes. ACh containing Ringer's solution was again applied. Reading from bottom to top: mechanical responses of the PRM to four successive ACh applications. Note the increase of sensitivity with repetitive applications of ACh (I–IV). The top trace shows the extracellularly recorded electrical activity accompanying the forth ACh contraction.

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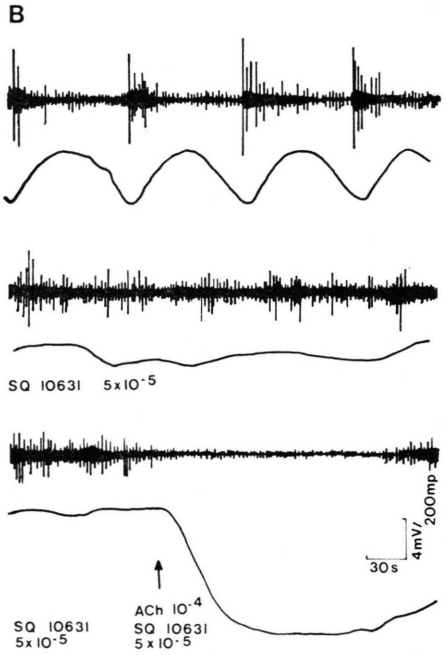
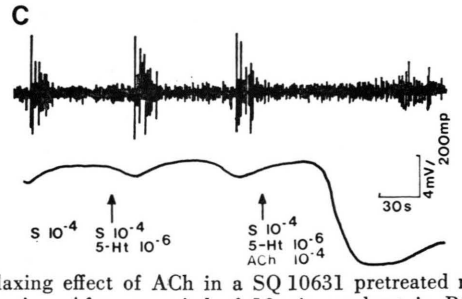


b. Effect of SQ 10631 on the spontaneous muscle activity. Upper panel: control; second panel: in the presence of SQ 10631; third panel: relaxation effect of ACh in SQ 10631 containing Ringer's solution. Note transient decrease of electrical activity (third panel upper trace) after ACh administration.



c. Relaxing effect of ACh in a SQ 10631 pretreated muscle preparation. After a period of 10 min washout in Ringer's solution containing Salyrgan (S) the relaxing effect of 5-Ht was prevented while the ACh evoked relaxation still occurred.

of ACh may be caused by presynaptic action of ACh on serotonergic relaxing nerve terminals also present in the isolated muscle preparations.

In this study, therefore, I would like to draw attention to the direct action of ACh on two representative gastropod smooth muscle preparations: the penis retractor muscle (PRM) and the pharynx retractor muscle of Helix pomatia. The 5-hydroxytryptamine (5-Ht) antagonist salyrgan[5] (10^{-4} M) was taken to eliminate postsynaptic effects of the biogenic amine possibly released *via* ACh-interaction with presynaptic cholinoceptive sites on serotonergic nerve terminals. Using coventional mechanical and electromyographic recording methodes[6] the response of the above mentioned muscle preparations to exogenously applied ACh was observed.

Fig. 1 depicts typical recordings which illustrated a dual effect of ACh on the PRM (the very similar results on the pharynx retractor muscle are not shown). While eliciting contraction on the one side (Fig. 1 a), ACh also inhibits spontaneously occurring rhythmic muscle activity and relaxes muscle tone (Fig. 1 b). These ACh induced inhibitory resp. relaxing effects took place after pre-exposure in 10^{-6} to 10^{-5} M 2-chloro-2′-(3-dimethylaminopropyl-thio) cinnamailide hydrochloride (SQ 10631), an ACh-antagonist, known to have a weak cholinergic blocking activity (*vs.* ACh) at concentrations of 2 to 8 μg/ml in rat uterus and guinea pig ileum *in vitro*. Since the participation of 5-Ht during the ACh evoked relaxation process was pharmacologically eliminated (Fig. 1 c) the finding confirmed an interaction of ACh with an ACh-receptor within the smooth muscle preparation which was unaffected by SQ 10631 and mediated relaxation. These data indicate that the action of ACh is not a simple one and that ACh is probably acting on more than one kind of receptor site within the muscle preparations.

One may conclude that in addition to excitatory ACh-receptors weakly antagonized by SQ 10631 the inhibitory action of ACh is brought about the result of the interaction of ACh with a second ACh-receptor type.

The observation that the relaxing response to ACh declines during the course of a treatment suggests that the preparation either metabolizes ACh or becomes desensitized to it. Since successive treatments with ACh show gradually decreasing inhibitory responses, the decline may be due to desensitization. The more so, because after pre-exposure in eserine, a potent anticholinesterase[3], a similar decline in the ACh-induced relaxing effect could be observed.

On the other hand, there is good reason to suppose that in the continued presence of ACh, in contrast to vertebrate skeletal muscle, the excitatory cholinoceptive sites show no desensitization (Fig. 1 a).

The present results summarized in Fig. 1 suggest that within the studied muscle preparations pharmacologically distinct. ACh-receptors coexist. Similar to other tissues with different kinds of cholinoceptive sites (*e. g.* molluscan neurons[7], frog lymph heart[8]) the response to exogenously applied ACh may be a multicomponent response (excitatory and inhibitory) in which the ACh evoked excitation naturally predominates. SQ 10631, a weak antagonist of the excitatory action of ACh was shown as a tool for studying the inhibitory action of ACh on molluscan smooth muscles.

The concept of different excitatory and inhibitory cholinergic receptor sites at the membrane of gastropod smooth muscles, supported by the present findings, may be useful to explain the response variety[9] of the gastropod smooth muscle preparations to exogenously applied ACh.

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