Positive and negative inotropic effects of carbachol on the embryonic chick atrium

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Received 6 January 1992; accepted 2 June 1992

Abstract. The effect of carbachol on twitch tension of atrial preparations from chick embryos of different incubation ages (3–14 days) was studied. At every age carbachol evoked negative (at low concentrations) and positive (at higher concentrations) inotropic responses. Maximal response values for both effects increased with age; in 3- and 5-day atria the positive inotropic response prevailed. The muscarinic antagonist pirenzepine inhibited the positive (on 5-day atria) and negative (on strips of 14-day atria) inotropic effects of carbachol with pA_2 values of 6.8 and 8.0, respectively, suggesting that muscarinic receptors mediating these effects belong to different receptor subtypes.
Key words. Carbachol; pirenzepine; muscarinic inotropism; atrium; chick embryo.

The negative inotropic effect of muscarinic stimulation has been observed in the embryonic chick heart as early as incubation day 3 [1]. Although these authors did not indicate of which heart chambers the preparation consisted, the response probably developed in the atrial tissue because the chick ventricle has been shown to be resistant to the direct inhibiting effects of muscarinic stimulation throughout embryonic development [2]. On the other hand, some facts indicate that muscarinic stimulation can also evoke a positive inotropic response in embryonic chick atria: a) such a response was shown in chicken atria pretreated with pertussis toxin [3], b) muscarinic stimulation evoked depolarization in the atrial cells of 3- and 4-day-old embryos [4].

The aim of the present study was to obtain direct evidence for both types of responses to muscarinic stimulation in embryonic chick atria, to follow any changes in these responses in the course of embryonic development, and to give a minimal pharmacological characterization of the receptors involved.

Materials and methods

Fertilized eggs of White Leghorn chickens were kept in a humidified incubator at 37–38 °C until the experiment was performed. Embryos older than 3 days were decapitated. From 3-day-old embryos, a whole atrium was isolated by cutting along the atrio-ventricular border; with 5- and 7-day-old embryos, only the left (whole) atrium was taken. From older animals a strip of the left atrium was prepared by making two parallel cuts from the left atrio-ventricular border to the interatrial septum. The strips from 10-day-old embryos were 2 × 0.5 mm, those from 14-day-old embryos 2.5 × 0.7 mm. Each preparation was placed between two platinum electrodes in a 10-ml bath containing a solution of the following composition (mM): NaCl 141, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 1.0, $NaHCO_3$ at a concentration sufficient to obtain the pH 7.2–7.4, glucose 5.5. The solution was bubbled with a mixture of 95% O_2 and 5% CO_2 at 30 °C. A preparation was connected to a force transducer made using the resistance strain gauge of KTD-7 type (Soviet production). The passive tension applied to the whole atrium from 3-day-old embryos was 1 mg; for atrial strips from 14-day-old embryos it was 5–8 mg. Preparations were driven with slightly suprathreshold rectangular electrical pulses of 1 ms in duration with a frequency of 1.3–1.8 Hz. Signals were recorded with KSP-4 (USSR) and Endim (Germany) pen recorders. The muscarinic agonist carbachol was added in increasing concentrations discretely or cumulatively until a maximal response was reached. Positive and negative inotropic responses (increase and decrease in twitch tension) were expressed as a percentage of the control twitch tension. Concentration-response curves for carbachol were constructed and EC_{50} values (concentrations at which carbachol produced a half-maximum response) were found. Antagonists (atropine and pirenzepine) were added at least 40 min prior to carbachol administration. The pA_2 values for antagonists were calculated from the equation $A_2 = B/(CR-1)$, where B is the concentration of an antagonist and CR is the ratio of EC_{50} for carbachol in the presence of antagonist to that without antagonist. All values are shown as arithmetic means ± SE. Statistics were performed using Student's t-test with a significance level of $p < 0.05$. The drugs used were carbachol chloride, atropine sulfate (both Soviet production) and pirenzepine dihydrochloride (Sigma).

Results

At all ages studied (3–14 incubation days) carbachol evoked a decrease as well as an increase of twitch tension (fig. 1). At low concentrations of carbachol (10–100 nM) only the decrease in twitch tension was observed, whereas at higher concentrations carbachol produced biphasic responses; a decrease followed by an increase in twitch tension. The time needed for a positive inotropic response to develop fully increased steadily during the first 10 days of incubation age (from 2.8 ± 0.1 min on 3-day atria to 6.5 ± 0.5 min on 10-day atrial strips), but increased sharply on the 12-day atrial strips (13.0 ± 1.0 min) when it was twice as much as on the 10-day strips ($p < 0.02$) (see also fig. 1C, D).

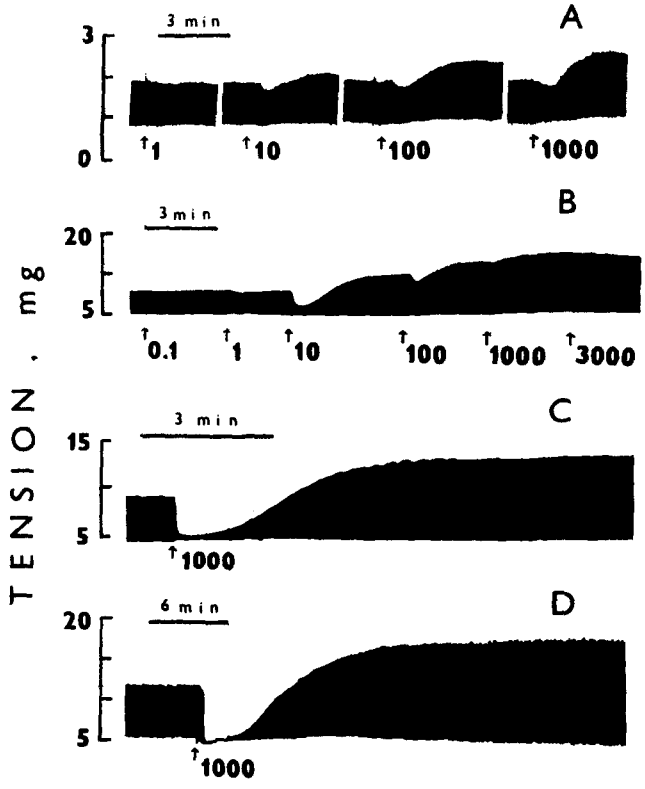

Figure 1. Inotropic effects of carbachol on electrically driven (1.3–1.8 Hz) embryonic chick atrial preparations. *A–D* Experimental records of twitch tension developed by whole atrium from 3-day-old embryo (A) and atrial strips from 10-day (B and C) and 14-day (D) embryos. Arrows indicate the addition of carbachol into the bath, numbers show final concentrations of carbachol in the solution ($\times 10^{-7}$M). Note the different magnitude of the negative inotropic response when the drug was added cumulatively (B) and discretely (C), and difference in time necessary for development of the positive inotropic response in (C) and (D).

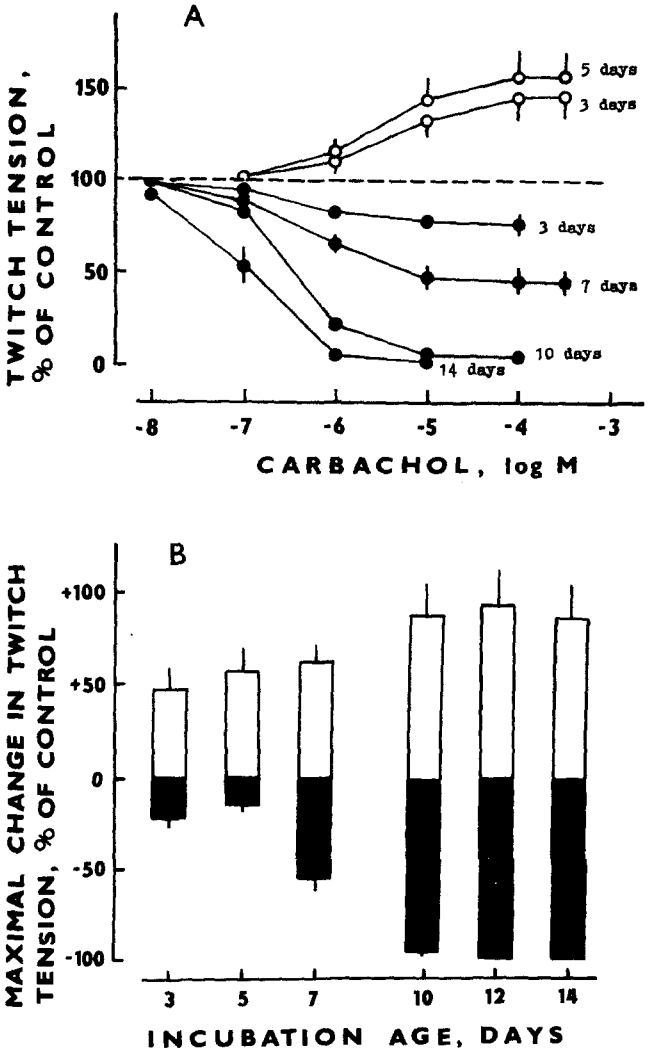

Figure 2. Concentration-dependence (A) and maximal response values (B) for inotropic effects of carbachol at different incubation ages. Light and black symbols = positive and negative inotropic effects, respectively. Each point or column is a mean; n = 5 or more except for 12 days (B), where n = 3. Vertical lines indicate SEM.

When carbachol was applied by discrete addition, the negative inotropic responses were somewhat greater than those obtained with cumulative addition (exceptionally, in 12- and 14-day preparations the responses were the same with both types of addition). Thus, when negative inotropic responses were measured, the 'discrete addition' method was used. For the same reason the 'cumulative addition' method was used to evaluate positive inotropic responses. Only one cumulative curve was obtained from each preparation. Figure 2 illustrates the concentration-dependence of both effects of carbachol, and gives maximal response values. In 3- and 5-day atria the mean maximal negative inotropic response did not exceed 23% and 16%, respectively. Two days later it became 3 times greater ($60 \pm 7\%$, $p < 0.05$), while on 10-, 12-, and 14-day atrial strips the maximal response value was near 100%. The EC_{50} value did not significantly change during the first 10 days of incubation ($0.28 \pm 0.10\,\mu M$, $0.50 \pm 0.10\,\mu M$, and $0.33 \pm 0.05\,\mu M$ on 3-, 7-, and 10-day atrial preparations, respectively); after that the EC_{50} value decreased and on 14-day strips it was found to be 3 times less than on 10-day strips ($0.11 \pm 0.03\,\mu M$, $p < 0.02$). The mean maximal positive inotropic response increased during the first 10 days of incubation ($47 \pm 12\%$ and $90 \pm 18\%$ for 3- and 10-day preparations, respectively) but the difference was not significant ($p < 0.1$). EC_{50} values were $4.9 \pm 1.1\,\mu M$, $4.3 \pm 0.9\,\mu M$, and $3.5 \pm 0.7\,\mu M$ on 3-, 5-, and 7-day atrial preparations, respectively. The values for older ages are not given because the dependence of the positive inotropic response on the concentration of carbachol could be influenced by the powerful negative inotropic effect occurring at this period of development.

The ability of both atropine (0.1 μM) and pirenzepine (1 μM) to antagonize the positive inotropic effect of carbachol was studied on 5-day atria, since the effect was found to be minimal at this age. Both antagonists evoked a parallel rightward shift in the concentration-response curve for carbachol (not shown) without significant changes in maximal response value. EC_{50} values for carbachol obtained in the presence of atropine and piren-

zepine (420 ± 70 μM and 41 ± 5 μM, respectively) significantly differed from that obtained without antagonists. pA_2 values were found to be 9.0 and 6.9 for atropine and pirenzepine, respectively. The potency of the antagonists' action against the negative inotropic effect of carbachol was studied on 14-day atrial strips. The concentration-response curves for carbachol were obtained before and after administration of an antagonist, and individual pA_2 values were found for each experiment. The mean pA_2 values were 9.1 ± 0.1 (n = 5) and 8.0 ± 0.1 (n = 6) for atropine and pirenzepine, respectively.

Discussion

The results of the present study show that muscarinic stimulation of the atrial tissue of 3–14-day-old chick embryos can result in both negative and positive inotropic responses. In 10–14-day atrial strips both responses were strongly developed, while in 3- and 5-day atria the positive response was relatively more developed than the negative one. It might indicate that in chick embryonic development the mechanism for positive inotropic responses to muscarinic stimulation appears earlier than the mechanism for negative responses. As shown by Löffelholz and Pappano [5], sensitivity of the embryonic chick heart to the negative chronotropic effect of acetylcholine increased on the 10th embryonic day, when cholinergic transmission could be detected in the heart. It is not unlikely that the prolonged negative inotropic reaction to carbachol and 3-fold increase in atrial sensitivity, which occurred in our experiments with embryos more than 10 incubation days old, are also related to the beginning of functional innervation of the heart.

Carbachol evoked the positive inotropic response at concentrations 5–10 times greater than those necessary for eliciting the negative inotropic response. Relatively high agonist concentrations are needed for the muscarinic positive inotropic response in adult heart as well as for the activation of phosphoinositide hydrolysis which is thought to participate in the mechanism for this response [3, 6, 7]. It should be noted, however, that concentrations of carbachol which appeared to be effective in the present study were 5 times less than those given in the reports cited.

It has been shown in the present study that the receptors mediating the negative inotropic response to carbachol have a high affinity for pirenzepine ($pA_2 = 8.0$); this value is close to that obtained on chicken atria ($pA_2 = 7.76$) [8]. At the same time the pA_2 value of pirenzepine's inhibition of the positive inotropic effect of carbachol was 10 times lower ($pA_2 = 6.9$). The difference in the affinity for pirenzepine strongly indicates that muscarinic receptors mediating the two opposing inotropic effects of carbachol belong to different receptor subtypes.

Twitch tension of atrial strips of 10–14-day embryos could be fully inhibited as well as markedly increased by muscarinic stimulation. This suggests that, at least during this period of development most (if not all) atrial cells possess the mechanisms for the two opposing responses.

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0014-4754/92/101007-03$1.50 + 0.20/0