J. Pharm. Pharmac., 1976, **28**, 196–199 *Received June* 26, 1975

Acetylcholinesterase and responses to acetylcholine in the embryonic chicken heart

J. LELORIER* AND F. E. SHIDEMAN

Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota, U.S.A.

Three and 4 day old embryonic chicken hearts were examined for their responsiveness to acetylcholine and presence of acetylcholinesterase (AChE) to determine the role of the enzyme in the cardiac effects of the transmitter. The effects of acetylcholine on rate and contractility of 3 day old hearts were indistinguishable from those on 4 day old hearts. These effects were readily blocked by atropine at both stages of development. In 3 day old hearts the responses to acetylcholine were not affected by the AChE inhibitor physostigmine but in 4 day old hearts they were considerably potentiated. The effect of acetylcholine on the rates of 4 day old hearts is of short duration (5 min or less). In 3 day old hearts it persists for a much longer time. Thus, the appearance of AChE in the embryonic heart of the chicken does not seem to modify the responsiveness of the cholinergic receptor to the transmitter.

Since it was first suggested that the enzyme acetylcholinesterase (AChE) might also function as the cholinergic receptor (Roepke, 1937), numerous conflicting data have appeared with respect to this hypothesis. Some of the supporting evidence includes the following. The change in kinetics of muscle AChE produced by denervation parallels the tendency of the muscle to develop contractures and its higher sensitivity to acetylcholine (Zupancic, 1953). Wurzel (1959), utilizing a series of choline esters, demonstrated the existence of a parallelism between their effects on a variety of organs and their rates of enzymatic hydrolysis by choline esterases. Detailed experimental analysis of the two systems, drug-AChE and drug-receptor, has shown them to be strikingly similar in their physicochemical and thermodynamic characteristics (Belleau, 1967). Ehrenpreis (1967) also has accumulated data which he has indicated do not prove AChE is the cholinergic receptor yet which he finds difficult to interpret otherwise.

Since earlier studies (McCarty, Lee & Shideman, 1960) suggested responsiveness of the developing heart of the embryonic chick occurred before the appearance of AChE, it was thought this preparation might serve as a model to determine the role played by the enzyme in responses to acetylcholine. The existence of full responsiveness of the heart to acetylcholine before appearance of AChE would indicate that the presence of the enzyme is not an essential component of the receptor. Simultaneous appearance of the enzyme and responsiveness to acetylcholine would neither prove nor disprove the essentiality of the enzyme for the receptor-response mechanism.

MATERIALS AND METHODS

Fertilized eggs, from white Leghorn chickens were incubated at 37° for the desired number of days. After removal, the embryos were placed in a warm (37°) Tyrode

*Present address: Department of Pharmacology, Medical School, University of Montreal, Montreal, Canada.

solution, gassed with 5% CO_2 in oxygen, having the following composition (g litre^{-1}): NaCl, 7·000; KCl, 0·354; $CaCl_2.2H_2O$, 0·350; KH_2PO_4, 0·081; $MgSO_4.7H_2O$, 0·147; $NaHCO_3$, 2·100; glucose, 0·900. Under a stereoscopic microscope, the hearts were carefully freed of other tissue and transferred to a bath containing 50 ml of Tyrode solution, aerated by the gases passing through a sintered glass plate in the bottom, and maintained at 37°. The hearts were impaled on two hooks prepared by drawing Pyrex glass rods of 5 mm diameter to a fine point. The lower hook was attached to a micrometric screw which was used to control the tension. The other hook was attached to a Hewlet-Packard 595 DT-005 Linearsyn differential transformer mounted on an LVDT transformer assembly (Gilson Medical Electronics). The output of the transformer was amplified by means of a Gilson CH-LVDT preamplifier and displayed on a Gilson M5P polygraph. This method, modified from that previously described (McCarty & others, 1960), permitted the recording of both rate and contractility.

Drugs, dissolved in Tyrode solution, were always added to the bath in a volume of 1 ml. Drug concentrations are expressed as final concentrations in bathing fluid. Unless otherwise noted, changes in heart rate after acetylcholine were determined by counting the number of beats during a 30 s interval, beginning 15 s after the addition of the drug. Changes in contractility were determined by comparing the maximal change in recorded amplitude after addition of the drug to the amplitude recorded during the 5 min period just before exposure to the drug. Both changes in rate and contractile amplitude are expressed as a percent of control values. Since complete recovery did not occur after exposure to the higher concentrations of acetylcholine, each heart was exposed only once to the drug. When either atropine or physostigmine was employed hearts were exposed to the drug for 10 min. This was followed by 3 washes with Tyrode solution, containing no drug, before addition of acetylcholine.

RESULTS

Preliminary experiments established that embryonic hearts begin to respond to acetylcholine on the third day.

At the concentrations of acetylcholine employed, the responses of 3 day old hearts are not significantly different from those of 4 day old hearts. This is evident from the dose-response curves illustrated in Fig. 1. The data in Fig. 2 show that the muscarinic

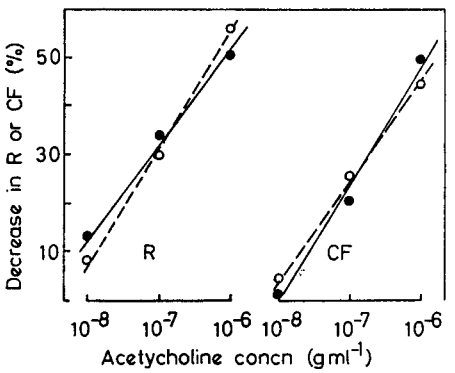

FIG. 1. Responses of 3 (○ – – ○) and 4 (●—●) day old embryonic chicken hearts to acetylcholine. Responses of hearts from the two age groups to a given concentration of acetylcholine are not significantly different ($P>0·05$). n = 5. R–rate, CF–contractile force.

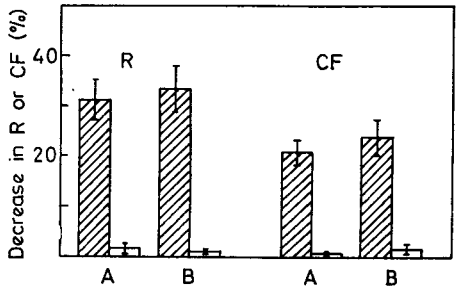

FIG. 2. Inhibition of responses to acetylcholine by atropine in 3(A) and 4(B) day old embryonic hearts. Hatched columns 10^{-7} g ml^{-1} acetylcholine. Open columns 10^{-7} g ml^{-1} acetylcholine after 10^{-4} g ml^{-1} atropine. Each vertical bar is the s.e.m. n = 6. R–rate, CF–contractile force.

blocking agent, atropine sulphate, at 10^{-4} g ml^{-1}, completely prevented the decreases in rate and contractility produced by acetylcholine 10^{-7} g ml^{-1}. The same degree of blockade occurs in hearts of both ages. However, while 3 and 4 day old embryonic hearts behave alike with respect to their responses to acetylcholine and the effects of atropine on these responses, they differ markedly in their responses to acetylcholine after pretreatment with physostigmine. Fig. 3 illustrates the results of the latter experiments. In 3 day old hearts the responses to acetylcholine are not significantly affected by pretreatment with physostigmine while in 4 day old hearts they are markedly potentiated. Recovery from the responses to acetylcholine (Fig. 4) is also different in 3 and 4 day old hearts. Five min after acetylcholine the rates of the 4 day old hearts returned to control levels while those of the 3 day old hearts remained depressed.

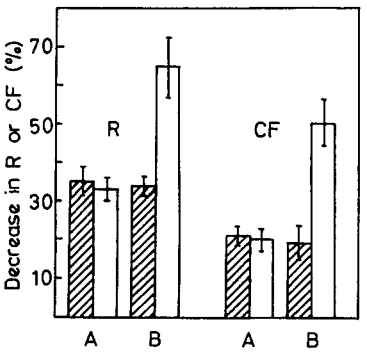

FIG. 3. Effects of physostigmine on responses to acetylcholine in 3(A) and 4(B) day old embryonic hearts. Hatched columns 10^{-7} g ml^{-1} acetylcholine. Open columns 10^{-7} g ml^{-1} acetylcholine after 10^{-6} g ml^{-1} physostigmine. Each vertical bar is the s.e.m. n = 6. R–rate, CF–contractile force.

DISCUSSION

The results presented can be considered under two headings. The first of these relates to a lack of difference in the magnitude of the inotropic and chronotropic responses to acetylcholine in 3 and 4 day old embryonic hearts. The second relates to the presence of AChE in hearts of 3 and 4 day old embryos.

The experimental evidence indicates that the cholinergic responses are of the same magnitude in 3 and 4 day old hearts (Fig. 2). It would be difficult to conceive of a

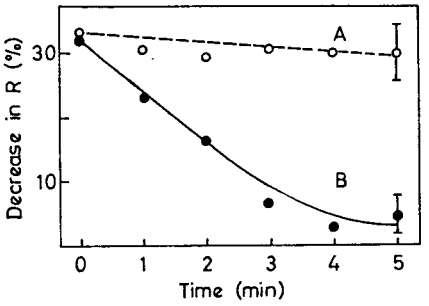

FIG. 4. Duration of the negative chronotropic response to acetylcholine (10^{-7} g ml^{-1}) in 3 (○ - - ○) and 4 (●—●) day old embryonic hearts. Each point on the curve is a mean of 5 experiments. Each vertical bar represents the s.e.m.

significant change in the structure or density of the cholinergic receptors occurring without altering the dose response curves. Hence, we believe these findings indicate that there is no significant difference in the nature of the cardiac cholinergic receptor at these two stages of development. Depression of cardiac contractility and rate can be produced by compounds acting through other than cholinergic mechanisms. This possibility can be ruled out by the observed blockage of responses to acetylcholine by the muscarinic blocking agent atropine (Fig. 3). The equally effective blockade by atropine in hearts of both ages militates against differences in the cholinergic receptors in these two groups.

While 3 and 4 day old hearts may be indistinguishable with respect to their responsiveness to acetylcholine, the remainder of the data presented indicate the existence of a marked difference with respect to the enzyme AChE. Physostigmine, a potent inhibitor of AChE, exerts its characteristic potentiation of the response to acetylcholine in 4 day old hearts. This is not the case, however, with 3 day old hearts where the inhibitor was without effect. The most plausible explanation for this observation is the absence of the enzyme at this state of development. Also, the brief duration of action of acetylcholine in the 4 day old as compared with that in the 3 day old heart is consistent with the appearance of a marked increase in activity of the enzyme between the 3rd and 4th days of embryological development.

In spite of the apparent close anatomical and functional association which exists between AChE and acetylcholine receptor, proof of their identity is lacking. The evidence derived from these studies militates against such identity since full responsiveness of the embryonic heart to acetylcholine is observed at a time when little or no AChE can be demonstrated to be present.

Acknowledgements

This research was supported in part by Grant No. HE 07939 from the National Institutes of Health, U.S. Public Health Service.

REFERENCES

BELLEAU, B. (1967). *Ann. N.Y. Acad. Sci.*, **144**, 705–719.
EHRENPREIS, S. (1967). *Ibid.*, **144**, 720–736.
MCCARTY, L. P., LEE, W. C. & SHIDEMAN, F. E. (1960). *J. Pharmac. exp. Ther.*, **129**, 315–321.
ROEPKE, M. H. (1937). *Ibid.*, **59**, 264–276.
WURZEL, M. (1959). *Experientia* (Basel), **15**, 430–432.
ZUPANCIC, A. D. (1953). *Acta physiol. scand.*, **29**, 63–71.