J. Physiol. (1977), **270**, *pp.* 271–282
With 7 text-figures
Printed in Great Britain

RAPID DESENSITIZATION OF ACETYLCHOLINE RECEPTORS OF EEL ELECTROPLAQUES FOLLOWING IONTOPHORETIC APPLICATION OF AGONIST COMPOUNDS

BY JOSÉ DEL CASTILLO AND GEORGE D. WEBB*

*From the Laboratory of Neurobiology and the Department of Pharmacology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico, 00905 and * Department of Physiology and Biophysics, University of Vermont College of Medicine, Burlington, Vermont 05401, U.S.A.*

(*Received 11 August 1976*)

SUMMARY

1. The electrical potential difference across the innervated membrane of the electroplaque of *Electrophorus electricus* was measured with an intracellular micro-electrode while an extracellular double-barrelled micropipette was used to apply acetylcholine and carbamylcholine iontophoretically very close to the point of insertion of the recording electrode.

2. The average depolarizing response to brief (several msec) pulses of carbamylcholine decayed 22 times more slowly than the response to acetylcholine. Treatment of the electroplaque with eserine or neostigmine prolonged the acetylcholine responses.

3. When a steady current of acetylcholine was applied for several seconds, the membrane first depolarized, then partially repolarized. Usually no repolarization was seen during long pulses of carbamylcholine or long pulses of acetylcholine in the presence of eserine or neostigmine.

4. During long conditioning pulses of acetylcholine or carbamylcholine, the responses to brief test pulses of acetylcholine showed a progressive decline in amplitude, but recovered after termination of the conditioning pulse. Desensitization half-times as short as 0·6 sec were observed, making these results similar to those obtained in the frog motor end-plate.

INTRODUCTION

In 1950 Fatt noted that when relatively high concentrations of acetylcholine (ACh) were applied to frog muscles, the end-plate regions became depolarized but then repolarized even though the ACh remained. Considerable washing was required to restore normal sensitivity. In 1955

Thesleff studied this phenomenon in more detail and found that it occurred with several cholinergic agonists. This apparent loss of sensitivity of the ACh receptors to the depolarizing action of cholinergic agonists has been termed desensitization (Katz & Thesleff, 1957). The mechanism for desensitization has not been clearly demonstrated.

Katz & Thesleff (1957) found that when ACh was applied to frog motor end-plates iontophoretically from a micropipette, desensitization occurred with a half-time as short as 1 sec. With bath application of cholinergic agonists, desensitization usually appears more slowly. When carbamylcholine (CCh) was bath applied to the eel electroplaque, desensitization developed with a half-time of about 1 min (Larmie & Webb, 1973*a*, *b*; Lester, Changeux & Sheridan, 1975), similar to the results obtained at frog motor end-plates (Thesleff, 1955). Presumably more rapid desensitization can be observed with the iontophoretic microtechnique because diffusion times are greatly reduced and because high agonist concentrations are reached.

We used the iontophoretic technique to see whether or not rapid desensitization, similar to that seen in frog muscle by Katz & Thesleff (1957), can be observed with electroplaque ACh receptors. As ACh receptor protein extracted from electric organs is widely used for receptor research, it is important to know whether or not this receptor has the same characteristics as the ACh receptor found in skeletal muscle. In this paper we will show that desensitization can be very rapid when CCh or ACh is applied to the electroplaque by iontophoresis. We will also show that the recorded responses to pulses of ACh differ from the responses to CCh, probably due to the action of ACh-esterase.

METHODS

The details of using the micro-electrophoretic technique with the electroplaque of *Electrophorus electricus* have been described by del Castillo, Bartels & Sobrino (1972). They pointed out that the depolarizations elicited by pulses of ACh and CCh cannot be recorded unless the tip of the recording micro-electrode is within a few μm of the tip of the drug micropipette, due to the short space constant of the electroplaque. Usually the recording micro-electrode was first inserted into the cell and then the tip of the double-barrelled drug micropipette was directed towards the recording tip at the bottom of the dimple created by the impalement. The tip of the drug pipette was usually less than 10 or 20 μm from the site of impalement. To exclude the possibility of electrical artifacts, a series of control experiments was run, during which long conditioning pulses of high-intensity current were passed through a saline filled barrel of a double-barrelled pipette, while periodic brief test pulses were being passed through the adjacent ACh barrel. Positive currents through the saline barrel had no significant effect on the responses to the ACh test pulses.

The physiological saline solution was based on an analysis of eel blood serum (Webb, Hamrell, Farquharson & Niemi, 1973) and had the following composition in mM: 188 NaCl; 5 KCl; 2 $MgCl_2$; 2 or 10 $CaCl_2$; 5 glucose and 1 Tris-(hydroxymethyl)-

amino-methane (adjusted to pH 7·4 with HCl). Experiments were done at 21–23° C. The ACh chloride and CCh chloride were obtained from the Sigma Chemical Co., St Louis, Mo. The eserine sulphate was from Mann Research Laboratories, New York, N.Y. Neostigmine bromide was from Hoffman-La Roche, Inc., Nutley, N.J. The concentrations of ACh and CCh in the drug micropipettes was 0·083 M for the former, and either 0·1 or 0·3 M for the latter.

All the results described below were repeated several times on electroplaques from two or more different eels.

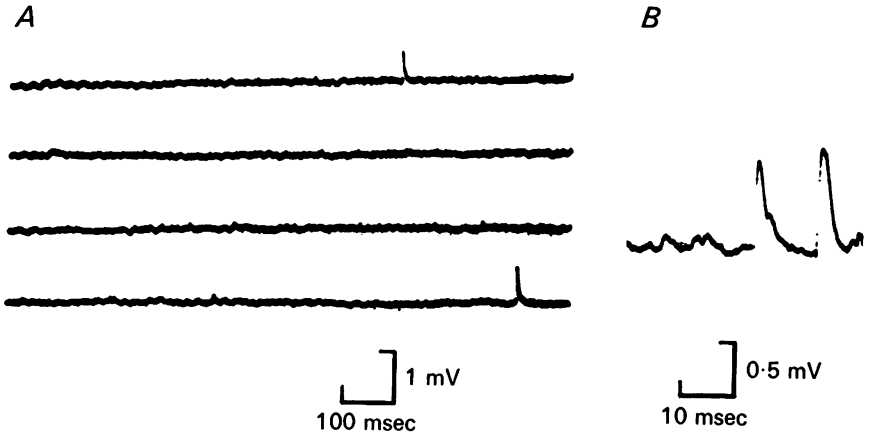

Fig. 1. Intracellularly recorded spontaneous miniature synaptic potentials. In *A* the resting potential was −86 mV and in *B* it was −72 mV. In *A* the trace was shifted down after each pass to show 4 continuous sec of time on one frame. In *B* (a different cell) the time scale is expanded to show the shape of two spontaneous potentials that came very close together.

RESULTS

Intracellularly recorded miniature synaptic potentials

As reported by del Castillo *et al.* (1972), spontaneous discharges similar to extracellularly recorded miniature 'end-plate' potentials were occasionally seen just before the tip of the recording micro-electrode penetrated the innervated membrane of the electroplaque. In addition, during the present experiments, brief spontaneous depolarizations were recorded intracellularly in two cells. Photographs of these intracellularly recorded miniature synaptic potentials are shown in Fig. 1.

The rarity with which miniature potentials are seen in electroplaques can be accounted for by the exceedingly short space constant of these cells. Since the resistance of the innervated membrane of the electroplaque may be as low as 1 Ω cm^2 (Nakamura, Nakajima & Grundfest, 1965), the calculated planar length constant may be as low as 20 μm (using the formula for a thick plane cell from Eisenberg & Johnson, 1970,

and assuming a cytoplasmic volume resistivity of 500 Ω cm). This agrees with our experimental results, as we found that no response to pulses of ACh could be observed unless the tip of the intracellular recording electrode was less than 10–20 μm from the extracellular ACh micropipette. ACh will, of course, cause the space constant to shrink due to the increased membrane conductance. Although there are thousands of synaptic junctions on each electroplaque, probably only 1 or 2 % of the innervated membrane is post-synaptic membrane. Only on those rare occasions when the tip of the recording micro-electrode happens to be placed in the immediate vicinity of a junction should one expect to see miniature synaptic potentials.

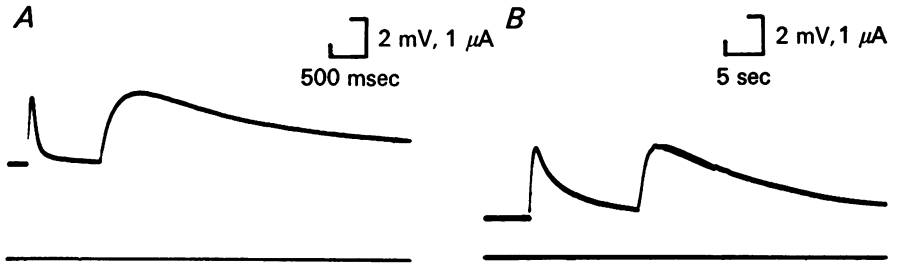

Fig. 2. The response to brief pulses of ACh and CCh before and after neostigmine. The resting potential was −74 mV and all of the pulse durations were 7 msec. In both *A* and *B* a single pulse of ACh was delivered followed by a single pulse of CCh. In *A* the ACh pulse was 0·43 μA in amplitude and the CCh pulse was 0·15 μA in amplitude. In *B* the ACh pulse was 0·36 μA in amplitude and the CCh pulse was 0·39 μA. In *A* the cell was bathed in eel physiological saline. *B* is a record from the same cell, but the site of impalement was different, the oscilloscope time scale was compressed, and 2 μg neostigmine bromide was added per ml saline in the bath 36 min before the record was made. Similar results were also obtained when using eserine sulphate (2 μg/ml.).

Responses to brief test pulses of ACh and CCh

When the recording and drug micropipettes were properly positioned, phasic depolarizations were seen following the application of brief (7·5 msec or less) pulses of ACh or CCh. The ACh and CCh-induced potentials had different time courses. The CCh potentials consistently decayed more slowly than the ACh potentials in the same cell. Fig. 2A shows an ACh potential followed by a CCh potential. The drug pulses were delivered from adjacent barrels of the same micropipette and the durations of both pulses were identical: 7 msec. A similar difference in time course was observed with every micropipette tested and cannot, therefore, be attributed to an asymmetry in the two openings of the micropipette tips. In this experiment, the ACh potential decayed with

a half-time of 78 msec, whereas the half-time of decay of the CCh potential was approximately 2·2 sec.

In the presence of eserine or neostigmine (2×10^{-6}, w/v) the difference between the time courses of the ACh and CCh potentials was considerably reduced as shown in Fig.2 *B* (notice the compression of the time scale in Fig. 2*B* compared with 2*A*). In Fig. 2*B* the ACh potential decayed with a half-time of 3·0 sec, but the CCh potential was also somewhat slower than in *A*. The mean decay half-times ($\pm$ S.D.) for five experiments of this type were: for Ach $0{\cdot}09 \pm 0{\cdot}02$ sec before neostigmine and $2{\cdot}6 \pm 0{\cdot}5$ sec after neostigmine, and for CCh $2{\cdot}0 \pm 0{\cdot}5$ seconds before and $7{\cdot}6 \pm 4{\cdot}3$ sec after. Thus neostigmine slowed the ACh decay about 29×, whereas the CCh decay was only slowed by about 4×.

In all experiments it was necessary to increase the amplitude of the current for the CCh pulse after neostigmine in order to achieve the same amplitude of response. This was probably due to inhibition of ACh receptors by neostigmine, as it has been demonstrated that neostigmine reacts with the ACh receptor of the eel electroplaque (Bartels, 1968). Since more CCh was released from the pipette, a wider area of receptors was stimulated and it took longer for the recorded response to rise and decay. Nevertheless, neostigmine increased the decay half-time for the ACh responses by much more than it did for the CCh responses, in spite of the fact that the pulse currents for ACh were usually less after neostigmine than the control. Therefore these experiments suggest that the brevity of the normal ACh responses (as compared to the CCh responses) was due at least in part to the hydrolytic activity of ACh-esterase. The decay in the presence of neostigmine appears to be too slow to be accounted for solely by diffusional loss, but the 'dimple' created by the impalement restricts the diffusion pathway. Since the 'dimple' is sometimes as deep as 100 μm, diffusion times of 2 or more sec are not unreasonable.

Responses to prolonged pulses of ACh and CCh

When pulses of ACh lasting several seconds were applied, the resulting depolarization was not maintained. It reached a maximum from 1–3 sec after the beginning of the pulse and decayed steadily thereafter as shown in Fig. 3A, where it can also be seen that a second application of ACh following a short resting interval results in a smaller depolarization than that attained initially. The shape of these potential changes suggests the occurrence of receptor desensitization. However, if long pulses of ACh were applied in the presence of neostigmine the resulting depolarization continuously increased until it reached a steady level, as shown in Fig. 3*B*.

Long pulses of CCh of up to 30 or more sec gave rise to slowly increasing depolarizations which usually did not reach a steady state or show any

decline; a typical response is illustrated in Fig. 4*A*. The shape of the potentials elicited by such long pulses of CCh was not modified by the presence of neostigmine in the bath (see Fig. 4*B*).

It appears therefore that the potential changes resulting from the application of long pulses of cholinergic drugs are not suitable for deriving conclusions on receptor desensitization. In the absence of enzymic drug hydrolysis, desensitization of the receptors immediately adjacent to the tip of the drug pipette may be masked by the slow build-up of drug concentration at the slightly more distant receptors.

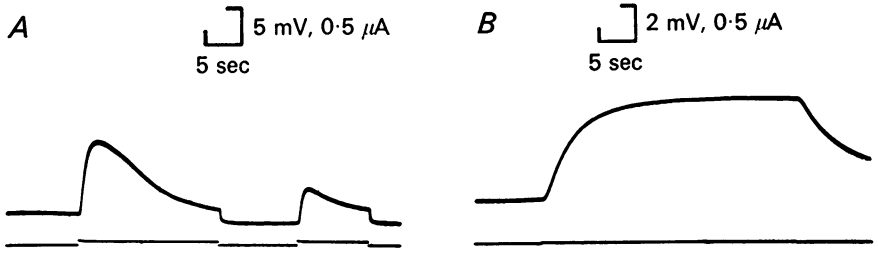

Fig. 3. The effects of prolonged pulses of ACh. In *A* the effects of two successive pulses of ACh are shown. In *B* the effect of a single long pulse of ACh is shown after the bath application of neostigmine (2 μg/ml.).

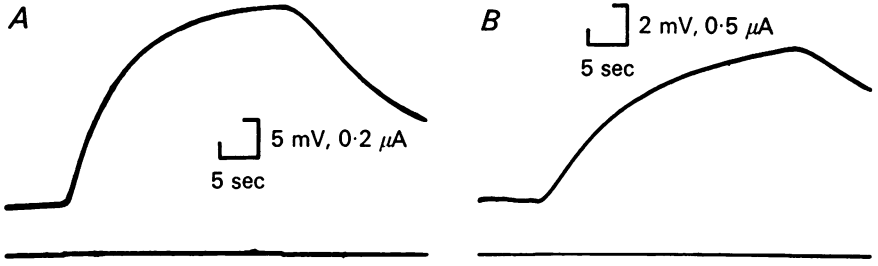

Fig. 4. The effects of prolonged pulses of CCh. *A* is without neostigmine and *B* is with 2 μg neostigmine/ml. of the bath. The difference in rise time was not consistent and was probably the result of different electrode placement.

Desensitization seen using brief test pulses and long conditioning pulses

Since brief drug pulses affect only the receptors very close to the tip of the drug micropipette, a much better way to assess receptor desensitization is to follow Katz & Thesleff's (1957) technique of applying brief pulses to test receptor sensitivity with one barrel of the pipette, and deliver longer 'conditioning' or 'desensitizing' pulses with the other barrel. Fig. 5*A* illustrates the results obtained with this procedure. In this instance, brief (5 msec) test pulses of ACh were applied at the rate of one every 1·3 sec and a long CCh conditioning pulse was delivered with the other barrel. In

this trial the response to the test pulses was reduced by one half after the conditioning pulse had been applied for about 0·6 sec.

A good way to show the time course of desensitization is to plot the amplitudes of successive test potentials as done in Fig. 5*B*, *C* and *D*, which show the time course of the receptor desensitization induced by conditioning pulses of decreasing strength. The half-times of desensitization were 0·6, 2·2 and 5·0 sec respectively for *B*, *C* and *D*. In Fig. 5*B*

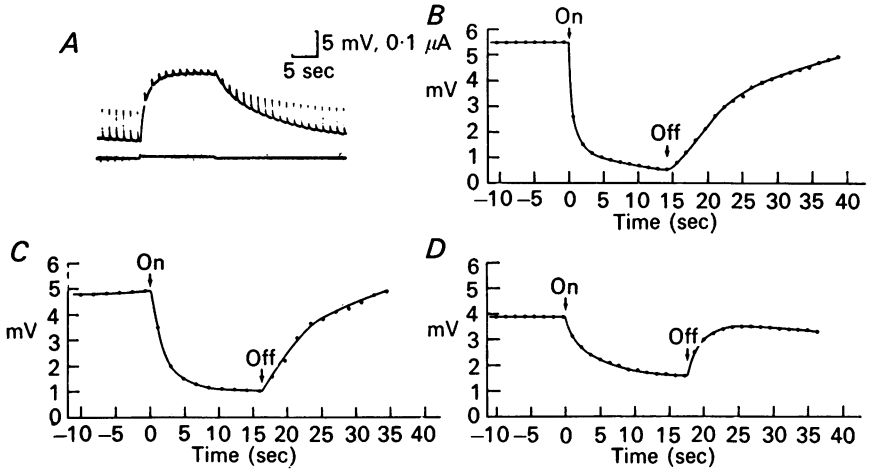

Fig. 5. Desensitization seen with brief ACh test pulses during prolonged (several seconds) conditioning pulses of CCh. *A* shows an actual record of a typical experiment. The resting potential was −77 mV; the test pulse duration was 5 msec and the amplitude was 0·24 μA. In this experiment and in the one plotted in *C* the Ca^{2+} concentration was 10 mM instead of the normal 2 mM. In *B* the data from *A* is plotted as the amplitude of the responses to the test pulses against time. *C* and *D* show other experiments plotted the same way. In *D* the maximum depolarization from the conditioning pulse of CCh was 7·4 mV as compared to the 13·2 mV seen in *A* (and *B*).

a maximal CCh depolarization of 13 mV abolished almost completely the responses to the test ACh pulses. This was followed by a slow recovery. In this experiment and in that illustrated in Fig. 5*C*, the Ca^{2+} concentration in the saline was increased from 2 to 10 mM. This seemed to increase the rate of desensitization, although not enough experiments were done to quantitate this effect. In Fig. 5*D*, the Ca^{2+} concentration was normal (2 mM) and the conditioning pulse was weak. Note the rapid recovery. The slower desensitization was probably due to a combination of the weaker CCh pulse and the lower Ca^{2+} concentration.

In Fig. 5*A* it can be seen that the CCh conditioning dose produced a greater depolarization than the test pulses. This raised the question as

to whether the decreased amplitude of the test pulses might have been due to a saturation of the receptors rather than to desensitization. In order to gain information that might help answer this question, experiments were run in which every other test pulse was about 3 times as large as the preceding or following one. The results are shown in Fig. 6. Note that the smaller pulses were affected by the conditioning pulse to the same extent as the larger test pulses. In eight experiments where 15 sec of a condition-

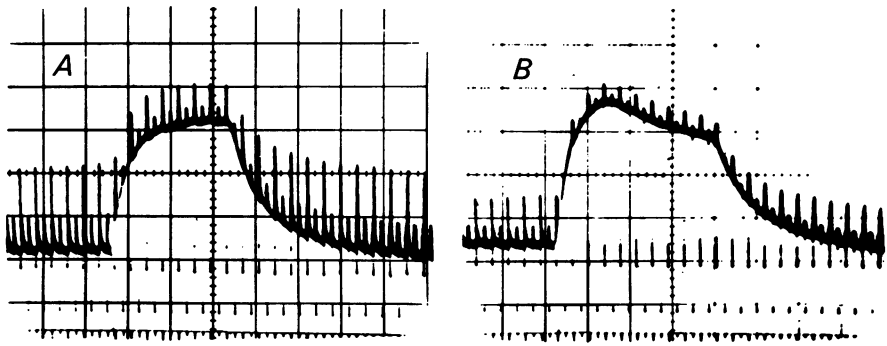

Fig. 6. Desensitization seen with variable sized test pulses of ACh during a CCh conditioning pulse. All test pulses were 7·5 msec in duration. The test pulse current amplitudes were constant throughout the experiment as in the previous experiments, except that every other pulse was about 3 times as large as the preceding or succeeding one. In *A* the resting potential was −83 mV, in *B* it was −82 mV. The scale is 5 sec/div. horizontal, and vertically the lower trace is 0·2 μA/div. and the upper trace is 4 mV/div.

ing pulse of CCh caused depolarizations from 6 to 22 mV, the average decrease of the test pulses (in % of the initial value) was 41 ± 13 % (S.D. of an observation) for the large test pulses and 41 ± 17 % for the small test pulses. If saturation of the receptors was beginning to occur, one would have expected the larger pulses to be reduced more than the smaller ones. These experiments suggest that saturation was not important under our experimental conditions. The only case where we observed a decrease in the amount of depolarization produced by a prolonged pulse of CCh occurred in the experiment shown in Fig. 6*B*. Perhaps in this one case the pipettes were near one small synapse, but all other synapses were too far away to be reached by CCh.

CCh could also be used to test receptor sensitivity, but was not as convenient as ACh due to the extremely slow rate of decay of the CCh potentials induced by brief pulses of this drug. An experiment in which a conditioning pulse of Ach was applied during a train of brief CCh test pulses is illustrated in Fig. 7*A*. Notice how the depolarization induced by the conditioning pulse decayed in the same manner as shown in Fig. 3*A*.

Fig. 7*B* shows a similar experiment performed in the presence of neostigmine in the bath (2×10^{-6}, w/v). The shape of depolarization produced by the conditioning ACh pulse has now changed.

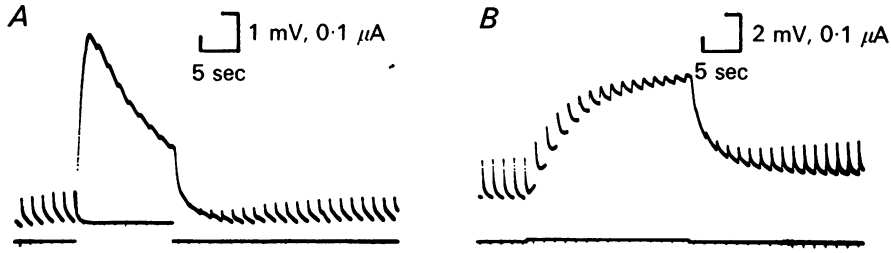

Fig. 7. Desensitization seen with brief CCh test pulses during a prolonged ACh conditioning pulse. The resting potential was −77 mV; test pulse duration was 5 msec. *A* is with the cell bathed in normal saline, *B* is with neostigmine added (2 μg/ml.) (same cell but different spot). In *A* the CCh test pulse amplitude was 0·43 μA and in *B* it was 0·04 μA.

DISCUSSION

The experiments described above have shown that when diffusion delays are eliminated by the use of micro-electrophoretic techniques, the cholinergic receptors of the electroplaque appear to desensitize much more rapidly than when the same drugs are added to the bath (Larmie & Webb 1973*b*; Lester, Changeux & Sheridan, 1975). In this respect the eel electroplaque appears to be very similar to the end-plates of the frog sartorius muscle. In the latter, with micro-electrophoretic application, the half-time for desensitization can be as short as 1 sec, depending on the strength of the conditioning pulses (Katz & Thesleff, 1957). In our experiments with the electroplaque we observed desensitization half-times as short as 0·6 sec. This is in contrast with desensitization half-times of many seconds to a minute seen when CCh is bath applied to the electroplaque (Larmie & Webb, 1973*b*; Lester *et al.* 1975; Pallotta, Webb & Sharp, 1976). Thus the ACh receptors are similar in this respect in both the electroplaque and the skeletal neuromuscular junction.

One important difference between frog end-plates and the innervated surface of the electroplaque is the extremely short space constant of the latter. In the frog the recording electrode can be over one mm away from the tip of the drug micropipette and still record responses to applied drugs. In the electroplaque, however, the tips of the recording and drug micropipettes must be within 10–20 μm of each other for any response to be recorded.

Nevertheless, long drug pulses probably allowed time for the CCh

concentration to build up gradually at the receptors located within the 20 to 40 μm diameter circle which the recording electrode could monitor, therefore desensitization occurring immediately under the drug pipette at the periphery of the circle was masked by activation of receptors farther away from the drug pipette. Desensitization of the receptors close to the drug pipette has the additional effect of extending the space constant due to the increased membrane resistance. The brief test pulses, on the other hand, produced significant drug concentrations only at those receptors immediately adjacent to the tip of the drug pipette, and these were the receptors which became desensitized to the long conditioning pulses. Therefore the test pulses allowed us to see desensitization occurring during a long conditioning pulse of CCh. Desensitization was observed during long conditioning pulses of ACh even without using test pulses, presumably because ACh-esterase prevented the ACh from diffusing very far from the drug pipette. In the presence of neostigmine, ACh behaved in much the same way as CCh.

Another result which suggested that ACh-esterase was very effective in removing ACh was the rapid decay of the response to brief pulses of ACh as compared with the decay of the CCh depolarizations. The half-time for the decay of a CCh potential was 22 times as long as the half-time for the decay of an ACh depolarization. Neostigmine reduced this difference between ACh and CCh responses to threefold. Similar results have been reported for frog sartorius end-plates (del Castillo & Katz, 1957), except that in the latter the decay of the CCh responses was only about twice as long as the decay of the ACh potentials (without neostigmine). This suggests that the ACh-esterase of the electroplaque is much more effective than that of the frog sartorius neuromuscular junction.

With regard to the question of whether or not receptors became saturated during these experiments, it should be pointed out that data of the type presented in Fig. 6 do not necessarily rule out the possibility of saturation. The ACh in the larger test pulses might have been able to diffuse farther than the ACh in the smaller ones before being hydrolysed. It is conceivable that saturation may have occurred very close to the pipette tips during a long conditioning pulse, but the larger test pulses might have remained proportionately larger than the smaller ones because of their larger diffusion area. Nevertheless, there is good evidence that regardless of whether or not saturation occurred, desensitization also occurred, since during a long conditioning pulse of ACh the response to the conditioning pulse itself declined. Presumably the responses to long CCh pulses did not show this decline because the CCh continued to diffuse out and activate more receptors (as did ACh in the presence of neostigmine).

In addition to saturation, other factors which may have caused a

reduction in the amplitude of the test pulses during a conditioning pulse are the voltage sensitivity of the activation response or the reduction in driving force as the reversal potential is approached. Both of these possibilities seem relatively unimportant, since apparent desensitization was seen even when the conditioning pulse produced less than a 2 mV depolarization. The largest conditioning depolarization used in the experiments illustrated was approximately 13 mV (from a resting potential of around −80 mV). This is still a long way from the reversal potential, which is about −4 mV for the electroplaque (Lassignal & Martin, 1976). Although activation of the ACh receptors of the electroplaque is extremely voltage-dependent as positive voltages are approached, the I–V curve in the presence of CCh is almost linear in the range from −80 to −60 mV (Ruiz-Manresa & Grundfest, 1971). It should be pointed out that the actual depolarization at the site of drug application was probably greater than what we measured, because of the short space constant. It is possible, therefore, that some portion of the apparent desensitization observed with the test pulses was due to the factors discussed above. The major portion of the apparent desensitization was probably due to classical desensitization, since when ACh was used for the conditioning pulse, the response to the conditioning pulse itself fell off at about the same rate as the response to the test pulses.

In conclusion, the desensitization of the ACh receptors in the electroplaque of *Electrophorus electricus* appears to be very similar to that described for the end-plates of skeletal muscle. In both preparations desensitization occurs within a few seconds when ACh or CCh is applied electrophoretically.

The authors wish to thank Mrs Nivia Deliz, Mr G. Garcia de la Noceda, Mr F. McKenzie and Mr H. Rodriguez for technical assistance. We also thank Drs Rodney L. Parsons and Vincent E. Dionne for their useful comments on the manuscript. This work was supported by U.S.P.H.S. grants nos. NS-07464 and 4-K6-N3-14, -938 to J. del C. and by a grant from the Muscular Dystrophy Associations of America to G.D.W. (Contribution no. 57, Laboratory of Neurobiology.)

REFERENCES

BARTELS, E. (1968). Reactions of acetylcholine receptor and esterase studied on the electroplax. *Biochem. Pharmacol.* **17**, 945–956.

DEL CASTILLO, J., BARTELS, E. & SOBRINO, J. A. (1972). Microelectrophoretic application of cholinergic compounds, protein oxidizing agents, and mercurials to the chemically excitable membrane of the electroplax. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2081–2085.

DEL CASTILLO, J. & KATZ, B. (1957). A comparison of acetylcholine and stable depolarizing agents. *Proc. R. Soc.* B **146**, 362–368.

EISENBERG, R. S. & JOHNSON, E. A. (1970). Three-dimensional electrical field problems in physiology. *Prog. Biophys. molec. Biol.* **20**, 1–65.

FATT, P. (1950). The electromotive action of acetylcholine at the motor end-plate. *J. Physiol.* **111**, 408–422.

KATZ, B. & THESLEFF, S. (1957). A study of the 'desensitization' produced by acetylcholine at the motor end-plate. *J. Physiol.* **138**, 63–80.

LARMIE, E. T. & WEBB, G. D. (1973*a*). Desensitization in the electroplax. *J. gen. Physiol.* **61**, 263.

LARMIE, E. T. & WEBB, G. D. (1973*b*). Transmembrane potential and resistance changes during desensitization in the electroplax. *Fedn Proc.* **32**, 333.

LASSIGNAL, N. L. & MARTIN, A. R. (1976). Reversal of acetylcholine potentials in eel electroplaque. *Science, N.Y.* **191**, 464–466.

LESTER, H. A., CHANGEUX, J.-P. & SHERIDAN, R. E. (1975). Conductance increases produced by bath application of cholinergic agonists to *Electrophorus* electroplaques. *J. gen. Physiol.* **65**, 797–816.

NAKAMURA, Y., NAKAJIMA, S. & GRUNDFEST, H. (1965). Analysis of spike electrogenesis and depolarizing K inactivation in electroplaques of *Electrophorus electricus*. L. *J. gen. Physiol.* **49**, 321–349.

NASTUK, W. L. & PARSONS, R. L. (1970). Factors in the inactivation of postjunctional membrane receptors of frog skeletal muscle. *J. gen. Physiol.* **56**, 218–249.

PALLOTTA, B. S., WEBB, G. D. & SHARP, R. W. (1976). Effect of ethanol, octanol, and pentobarbital on the rate of desensitization in voltage-clamped eel electroplaque. *Neurosci. Abst.* **2**, 705.

RUIZ-MANRESA, F. & GRUNDFEST, H. (1971). Synaptic electrogenesis in eel electroplaques. *J. gen. Physiol.* **57**, 71–92.

THESLEFF, S. (1955). The mode of neuromuscular block caused by acetylcholine, nicotine, decamethonium and succinylcholine. *Acta physiol. scand.* **34**, 218–231.

WEBB, G. D., HAMRELL, B. B., FARQUHARSON, D. A. & NIEMI, W. D. (1973). Studies of the blood chemistry of *Electrophorus electricus* and a new physiological saline solution based thereon. *Biochim. biophys. Acta* **297**, 313–316.