THE ACTION OF PROCAINE AT THE NEUROMUSCULAR JUNCTION

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The mode of action of procaine at the neuromuscular junction has been studied in the rat isolated phrenic nerve diaphragm preparation. Procaine had no effect on the response to indirect stimulation in concentrations below 8×10^{-5}. But 2×10^{-5} procaine reduced the neostigmine potentiated twitch to its normal size, and caused a significant reduction in the amount of acetylcholine released by a period of tetanus. This is thought to be due to a selective local anaesthetic action on the fine motor nerve terminals.

NUMEROUS reports have shown that procaine can cause neuromuscular blockade. This is usually attributed to its curare-like action (Fulton, 1921; Peterson, 1955), but qualitative differences between the action of curare and procaine at the neuromuscular junction, suggested that procaine might in addition interfere with the release of acetylcholine from motor nerve endings (Harvey, 1939; Jaco and Wood, 1944; Bülbring, 1946). The present experiments were undertaken therefore to see if this suggestion could be demonstrated by the direct measurement of acetylcholine release.

METHODS

The method for studying acetylcholine release has been described previously (Straughan 1959; 1960). A single side of a rat diaphragm with its attached phrenic nerve was suspended flat in a glass diaphragm bath containing 3 ml. of Krebs solution with 5×10^{-6} neostigmine methylsulphate. The nerve was stimulated with supramaximal rectangular pulses, 0·03 millisec. in duration for 20 min. periods at a rate of 25/sec. Immediately after the end of stimulation the bath fluid was removed for assay. Up to six successive periods of tetanus with 10 min. rest between them, can be applied to each diaphragm, and the acetylcholine release has been shown to be steady under these conditions. The temperature was kept constant at $37° \pm 0{\cdot}25°$ in each experiment.

Samples of bath fluid containing acetylcholine were assayed on the rat blood pressure preparation, this has been fully described previously (Straughan, 1958; 1959; 1960).

RESULTS

The effect of procaine on the response to indirect stimulation. A rat diaphragm was set up in the manner described by Bülbring (1946), in Krebs solution and stimulated indirectly through the phrenic nerve at 8/min. with supramaximal rectangular pulses. The addition of procaine to the bath fluid in concentrations 1×10^{-5}, 2×10^{-5} and 4×10^{-5} had no effect on the size of the muscle twitch. When the procaine

concentration was increased to 8×10^{-5} a small diminution in twitch height developed gradually over 10 min. Complete abolition of the twitch response to nerve stimulation developed over 10 min. at a procaine concentration of $1{\cdot}6 \times 10^{-4}$.

Concentrations of procaine which were without effect on twitch height caused an immediate reduction to its previous size of the twitch potentiated by neostigmine methylsulphate (1×10^{-6}). The same effect occurs with procaine and neostigmine in the cat gastrocnemius muscle (Jaco and Wood 1944). These observations are illustrated in Fig. 1.

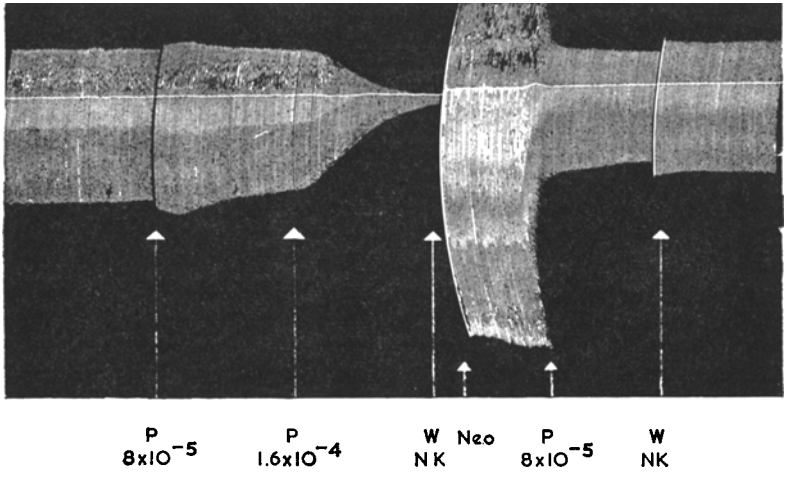

FIG. 1. The action of procaine on the indirect twitch before and after neostigmine. Rat isolated diaphragm preparation stimulated through phrenic nerve at 8/min. At P, procaine added to bath fluid for 10 min. At WNK preparation washed with plain Krebs. At Neo, neostigmine methylsulphate ($1 + 10^{-6}$) added to bath for 10 min.

The effect of procaine on the release of acetylcholine. A standard procedure was adopted; after immersing the preparation for 30 min. in 5×10^{-6} neostigmine methylsulphate, two successive periods of tetanus were applied and the bath fluid collected each time for assay. The diaphragm was now allowed to equilibrate for 15 min. to a known

TABLE I

THE EFFECT OF PROCAINE ON ACETYLCHOLINE RELEASE FROM THE RAT DIAPHRAGM
ACETYLCHOLINE IN NG. BASE/20 MIN. AT 25/SEC.

EACH FIGURE IS THE MEAN OF TWO SUCCESSIVE PERIODS OF STIMULATION

Expt. 1	No procaine	2×10^{-5} procaine	4×10^{-5} procaine
1	62	53	—
2	59	52	—
3	44	28	—
4	38	21	—
5	37	33	—
6	48	41	33
7	42	30	19
8	61	—	34
9	44	—	32
10	49	—	29

concentration of procaine, two further periods of tetanus were applied and the samples collected. This was repeated in a concentration of increased procaine. All the samples were assayed individually and a mean acetylcholine release figure calculated from the two values obtained at each particular concentration of procaine.

The results showed that even a concentration of procaine as small as 2×10^{-5} caused a significant reduction in the amount of acetylcholine released from the rat diaphragm preparation by a period of tetanus. Further increases in the procaine concentration brought about an even greater reduction in the amount of acetylcholine released, so that in one diaphragm the mean release in normal Krebs fluid was 64 ng. base/20 min. at 25/sec., the addition of procaine 4×10^{-5} now caused the mean release to fall to 37 ng. base/20 min. at 25/sec. and when the procaine concentration was increased to 8×10^{-5} the mean acetylcholine release fell even further, to 15 ng. base. The results from ten other typical experiments are shown in Table I.

Discussion

These results suggest that procaine in doses which have no curarising effect reduces acetylcholine release. This observation is supported by results reported by Harvey (1939). He observed that procaine was less curarising than tubocurarine, dose for dose, in that it reduced the twitch response of the cat tibialis muscle to injected acetylcholine less than did tubocurarine. A tetanus applied to the motor nerve produced a partial relief of the neuromuscular blockade produced by procaine, but exaggerated a tubocurarine block.

The observation that 2×10^{-5} procaine reduced the indirect twitch of the rat diaphragm only after it had been potentiated by neostigmine, can be explained as follows. Because of the high safety factor for neuromuscular transmission by acetylcholine, the reduction in release which follows small doses of procaine is insufficient to impair transmission. But, when neostigmine is present acetylcholine accumulates at the end plate, repetitive firing occurs, and the twitch height is potentiated. The reduced amount of acetylcholine now released after a small dose of procaine, is then just sufficient to maintain the potentiated twitch height at its former size.

It is clear from the results that procaine interferes with release during high frequency nerve stimulation. There are good reasons for believing that this action of procaine is a general one and not just due to the conditions of stimulation. Firstly, Furukawa (1957) showed by electrophysiological methods that procaine reduced the acetylcholine release in frog muscle even at low rates of stimulation. Secondly, the results suggest that the release from motor nerve endings in the rat diaphragm can be reduced by half without impairment of transmission as judged by the twitch response at low rates of stimulation. This is consistent with current knowledge of a high safety factor for neuromuscular transmission by acetylcholine. Kuffler (1942) showed that the end plate potential in

frog muscle could be reduced to 30 or 40 per cent of its original height before transmission failed. Thirdly the qualitative differences seen between curare and procaine at low rates of stimulation by Jaco and Wood (1944) were seen also at high rates of stimulation by Bülbring (1946).

It remains a possibility that high frequencies of stimulation exaggerate the action of procaine on acetylcholine release, perhaps by increasing the extent of presynaptic failure of nervous transmission already present under these conditions (Krnjević and Miledi, 1958).

The action of procaine on acetylcholine release may be attributed to its local anaesthetic properties, since cocaine also appears to reduce acetylcholine release in the rat diaphragm, Bülbring (1946). Harvey (1939) suggested that procaine might interfere with release by acting on the terminal branches of the motor nerve. This is the most likely site of action since the terminals are of fine diameter and are unmyelinated which should make them preferentially sensitive to the action of local anaesthetics (Gasser and Erlanger, 1929; Matthews and Rushworth, 1957).

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