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PHARMACOLOGICAL ACTIONS OF INJECTED CHOLINESTERASE*

BY

I. T. BECK

From the Department of Pharmacology, Faculty of Medicine, McGill University, Montreal (Quebec), Canada

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In a previous study (Beck, 1951), it was shown that intravenously injected " true " cholinesterase from bovine erythrocytes† (mentioned as red cell enzyme) was without perceptible effect upon neuromuscular transmission, but that the enzyme was active against intravenously-injected (circulating) acetylcholine. In view of the overwhelming evidence for the acetylcholine-cholinesterase system in neuromuscular transmission, no attempt was made to explain the negative results by questioning the importance of the enzyme. It was concluded that either (1) the injected enzyme did not reach the structures in the end plate where cholinesterase normally occurs and exerts its hydrolytic action, or (2) that some penetration occurred but that the excess of the enzyme at the vital points is normally so high that any increase in concentration which may have occurred was too small, relatively to the quantity already present, to be pharmacologically detectable.

The experiments presented here were carried out to throw some light on the latter possibility. It was thought that the action of the injected enzyme, if it could reach the point essential for its action even in minute quantities, would be easily detected if the amount of normal cholinesterase in the end plates had been reduced beforehand.

METHODS

The methods employed were those described in the first communication (Beck, 1951). The activity of the different batches of the cholinesterase obtained from Winthrop-Stearns varied from 33–110 per cent of the activity indicated on the vials (determined manometrically). Doses mentioned in this work are all corrected values; thus if the red cell enzyme had only 33 per cent of its stated activity, three times the regular dose was injected to obtain a 100 per cent dose value. Doses are expressed in units. One unit of the enzyme hydrolyses 8 μg. acetylcholine (base) under conditions described previously.

RESULTS

Action of the red cell enzyme on neuromuscular transmission in vivo

(1) *After di*isopropylfluorophosphate (DFP).—Male and female rats weighing around 300 g. were used. Because of the slow development of the action of intramuscularly injected DFP the substance (1 mg./kg.) was administered 5–6 hours before

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† Obtained from Winthrop-Stearns, Inc., Special Division, New York.

the beginning of the experiments.* According to Koelle and Gilman (1946), 5 hours after this dose the muscle cholinesterase of rats is depressed to 18 per cent of the original value and remains at this level for at least a day. Considering the experiments and calculations of Marnay and Nachmansohn (1938) and the histochemical experiments of Koelle (1950), it is assumed that the cholinesterase level *at the end plate itself*, at the moment of the experiment, was at about 18 per cent of the original level. The rats exhibited the symptoms described by Koelle and Gilman (1946), but generally the muscarinic symptoms prevailed over the nicotinic. At this stage the operation was carried out as described previously (Beck, 1951) for the experiments on the sciatic-gastrocnemius preparation of the rat. Sub- or supra-maximal stimuli were applied twice per minute. The intravenous injection of 330–4,000 units of the red cell enzyme into these DFP-poisoned rats was without any appreciable effect upon the twitch tension developed by the gastrocnemius muscle, nor did the enzyme diminish the intoxication symptoms (heavy breathing due to bronchoconstriction, muscular fasciculation, etc.). In some experiments (the tracing of one of these is reproduced in Fig. 1) a further injection of 1 mg. DFP per kg. was given intraperitoneally after previous atropinization (see legend of Fig. 1). Presumably the activity of the end plate cholinesterase was even lower than 18 per cent, though it was not diminished to such an extent as to cause failure of the neuromuscular transmission.

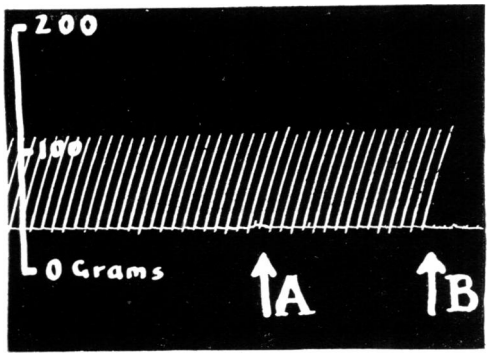

FIG. 1.—♀ Rat 255 g. Chloralose. Isometric recording of the gastrocnemius. Submaximal stimulation of the sciatic nerve. One stimulus every 30 seconds. The animal was injected at 9.35 a.m. with 1 mg./kg. DFP intramuscularly. The preparation for the experiment began at about 2.00 p.m. and was finished at 2.35 p.m. Between 2.35 and 2.45 p.m. 1,000 units of red cell enzyme was injected intravenously without producing any visible effect. At 3.00 p.m. 0.25 mg./kg. atropine sulphate was injected intravenously followed shortly by a second injection of 1 mg./kg. DFP, this time intraperitoneally. Twenty minutes after the injection of DFP a gradual increase of twitch tension developed, which is to be seen in the tracing shown above. Shortly before the part shown 330 units of the enzyme was injected without any visible effect. The beginning of the part shown began at 3.55 p.m. At A, intravenous injection of 1,400 units of the red cell enzyme; at B, intravenous injection of 10 mg./kg. Flaxedil.

Even at this very low level of cholinesterase activity the injection of the red cell enzyme was without any effect, though other drugs were able to exert their usual effect as proved by the instantaneous curarizing action of intravenously injected

* The DFP preparation was a 0.1 per cent solution in peanut oil (Floropryl Merck).

Flaxedil* (Tris (triethylaminoethoxy) 1–2–3 benzene), (Bovet, Depierre, and de Lestrange, 1947).

(2) *After neostigmine methylsulphate.*—The method was the same as in the previous section, with the exception that no pretreatment of the animal was given before the operation. The sciatic nerves of the rats were stimulated twice a minute sub- or supra-maximally. Fifty μg. neostigmine methylsulphate per kg. was injected intravenously. Once the increase of twitch tension, indicating the diminution of cholinesterase activity at the end plate, had developed and had become stabilized, different amounts of enzyme (up to 4,000 units) were injected intravenously. The lack of change in the contraction height of the muscle is well demonstrated in Fig. 2. The injection of Flaxedil on the end of each experiment served as a check upon the dependability of the preparation.

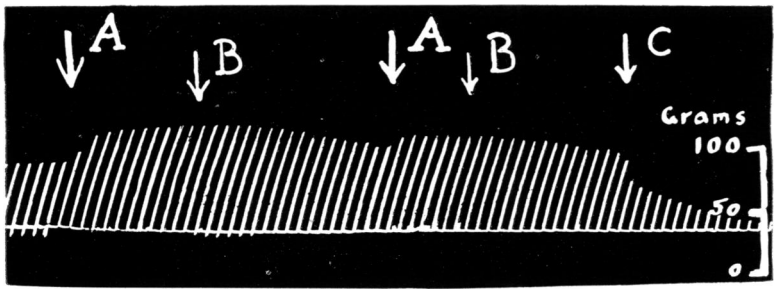

FIG. 2.—♀ Rat 270 g. Chloralose. Isometric recording of the gastrocnemius. Supramaximal stimulation of the sciatic nerve. One stimulus every 30 seconds. At A, 50 μg./kg. neostigmine methylsulphate intravenously; at B, 1,400 units of cholinesterase injected intravenously; at C, 2 mg./kg. Flaxedil intravenously.

The action of the red cell enzyme upon neuromuscular transmission in vitro *after the administration of di*isopropylfluorophosphate

Bülbring's (1946) preparation was used, modified as in the previous communication (Beck, 1951). Five hours before the dissection of the phrenic-diaphragm, the rats were intramuscularly injected with 1 mg./kg. of DFP. The addition of up to 5,000 units of the red cell enzyme to the isolated preparation suspended in 50 c.c. bath fluid was without any observable effect on contraction strength of the muscle.

DISCUSSION

The inability of bovine erythrocyte cholinesterase to produce a change in neuromuscular transmission when the cholinesterase activity of the end plate had been previously depressed by DFP or neostigmine proves that its failure to produce detectable effects in normal preparations was not due to the presence of a physiological excess of cholinesterase at the end plate.

It is well known that slight changes in the normal end plate potential are not detectable with mechanical registration. However, when cholinesterase activity is diminished by certain substances the end plate potential is greatly exaggerated

* Flaxedil was obtained from Poulenc, Ltd., Montreal.

(Eccles, Katz, and Kuffler, 1942), and a single induced stimulus produces a repetitive discharge. The increase in muscle tension is due to a tetanus instead of a single contraction. Even a *very slight* diminution of the end plate potential in a poisoned muscle would reduce the number of discharges, which in turn would diminish the muscle's contraction strength. If the injected cholinesterase could reach the place where acetylcholine is normally hydrolysed, this would be very easily demonstrated.

The results of these experiments seem to indicate that the failure of the red cell enzyme to produce changes in neuromuscular transmission in non-poisoned preparations is due to its inability to penetrate to those points where acetylcholine is destroyed after it has served its physiological function—the transmission of an impulse. Karczmar, Koppanyi, and Sheatz (1951) came to similar conclusions concerning sympathetic ganglia. However, the original cholinesterase activity of the sympathetic ganglia, in their experiments, was so high that minor changes would have been hardly detectable by the manometric method. Until it can be proved that the enzyme, when applied from the outside, reaches the crucial sites but still produces no effects, the importance of the acetylcholine-cholinesterase system in neuromuscular transmission cannot be doubted. The above experiments and those of Karczmar *et al.* are not the only proofs that cholinesterases circulating in the blood cannot enter the parts where acetylcholine is destroyed. There is, for instance, the simple and well-known fact that if DFP is locally applied to the eye the myosis and cholinesterase inactivation of iris and ciliary body endures for at least a couple of days (DeRoetth, 1951), although the cholinesterase activity of serum, and blood, is unaltered. This seems to indicate that tissue cholinesterase is not derived from blood cholinesterase but is produced locally, or moves into position from nerve fibres.

SUMMARY

Purified " true " cholinesterase from bovine erythrocytes is without mechanically detectable effect on neuromuscular transmission (rat sciatic-gastrocnemius preparation *in vivo* and phrenic-diaphragm *in vitro*) even if the animals are previously poisoned with anticholinesterase drugs.

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