THE EFFECT OF CENTROPHENOXINE AT THE SKELETAL NEUROMUSCULAR JUNCTION

S. D. MISTRY*, C. G. TRIPATHI, V. H. BHAVSAR AND V. V. KELKAR

Department of Pharmacology,
Government Medical College, Surat - 395 001

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Summary : Centrophenoxine exhibited some interesting actions at the neuromuscular junction. The drug was ineffective in rat or chick preparations, but blocked neuromuscular transmission in frog preparations. The blockade was reversed by adrenaline, potassium, choline and physostigmine. The drug had no effect on muscle contractility or endplate cholinoceptor. Hemicholinium 3 induced a neuromuscular blockade in rat (*in vivo*) which was reversed by choline but not by centrophenoxine. Neither of these two drugs could reverse the blocking effect of hemicholinium in frog preparations. It is concluded that centrophenoxine acts only in frog and the blockade involves a presynaptic mechanism. The work further suggests that choline uptake systems in the rat and the frog may not be identical, since choline competed with hemicholinium for the uptake system in rat and with centrophenoxine (but not with hemicholinium) in the frog.

Key words : Centrophenoxine skeletal neuromuscular junction hemicholinium frog sartorius muscle

INTRODUCTION

Centrophenoxine (meclophenoxate, deanol ; CP), a close structural analogue of choline, is a cerebral stimulant of some clinical utility in mental retardation in children, mental confusion in elderly patients, asphyxia neonatorum, delayed recovery from anaesthesia (9) and in tardive dyskinesias that follow the chronic use of neuroleptics (4, 7). Although the drug produces behavioural signs of excitation (6) and increases serum choline levels (10), the effect is probably unrelated to its conversion to acetylcholine (ACh) in brain (6). In view of this we investigated on peripheral action of CP at the skeletal neuromuscular junction, an aspect which has received little attention.

MATERIAL AND METHODS

Amphibian muscle and nerve-muscle preparations

Frog nerve-sartorius muscle preparation : The preparations made from Rana pipiens were set up in well aerated 30 *ml* baths in frog-Ringer solution at 22°–24°C for

*Corresponding Author

the indirect and direct stimulation (supramaximal shocks, 0.5. and 5 msec respectively, once every 10 sec). The twitch responses were recorded semiisometrically with a spring-loaded lever (magnification, x 8) putting the tissue under 0.6 *g* tension. Swing of the lever was calibrated in the range of records to measure tension (g). Reproducible responses could be obtained for 3 to 4 hr if the stimulation period (20 min) was following by a wash and rest for 5 min. To characterize a blocking effect, the latency period, $T\frac{1}{2}$ (the time to half decay of twitch tension) and the magnitude of blockade (% of control) were studied from a plot of twitch tension (semilogarithmic scale) against time (min) as described by Freeman (5).

Frog rectus abdominis muscle : The recti of Rana pipiens were set up in pairs (see above). The tone was recorded with isotonic levers (magnification, x 8) putting tissues under tension of 2 g. All preparations were stretched *in vitro* for 2 hr prior to the work.

Concentration-effect curves for ACh were elicited by adding increasing doses of the agonist at 7 min intervals. The effect (% maximum) was plotted against the concentration (M) after due compensation for spontaneous change in tissue sensitivity (as adjudged from the companion control preparation). The curves were recorded before and after addition of CP to the bath.

In some experiments, pA_2 value of tubocurarine was determined as described by Ariens *et al.* (7). The value was determined again in presence of CP after the preparations recovered from earlier curare-blockade. The values were compared using paired t-test.

In another series of experiments, the recti were set up for field stimulation (submaximal shocks, 20 msec. once every 20 sec). The responses were reproducible if stimulation period (15-20 min) was followed by a wash and rest for 10 min.

Mammalian and avian nerve-muscle preparations

The rat phrenic nerve-diaphragm and chick biventer cervicis preparation : The former were set up in Tyrode solution at 37°C, gassed with 5% CO_2 in O_2. The nerve was stimulated by supramaximal pulses (0.2 msec) every 10 sec. Biventer cervicis of 10 days old chick was set up in oxygenated Tyrode solution at 40°C. Ring electrodes were placed around the tendon for stimulation (supramaximal shocks, 0.5 msec, once every 10 sec). In both cases the contractions were recorded by spring-loaded levers (see above).

The rat sciatic nerve-gastrocnemius muscle preparation (in vivo) : Male albino rats (250-300 g) were anaesthetized with urethane (1.25 g/*kg*, ip) after premedication with hyoscine (1 *mg/kg*, ip). Rectal temperature was maintained at 37°C throughout the experiments. Trachea was cannulated for positive pressure ventilation with

air (8 *ml*/100 g, 50/min). The right thigh was fixed in vertical position by a metal pin passed through the lower end of femur. The gastrocnemius tendon was tied to an isometric lever (magnification, x 12 ; resting tension on muscle, 10 g) writing on smoked kymograph paper. Sciatic nerve (well up in thigh) was stimulated through a pair of platinum electrodes (supramaximal shocks, 0.5 msec, once every 10 sec). Drugs were injected through a cannulated external jugular vein.

Drugs

Drugs used were acetylcholine chloride, physostigmine salicylate, choline chloride, potassium chloride, hyoscine hydrobromide, and centrophenoxine hydrochloride (Lucidril, Laboratoires Anphar, Spain). Doses refer to the salts. (±)-Adrenaline base Hemicholinium 3 (HC 3 ; Aldrich Chemical Co., Milwaukee) were made up fresh in distilled water before use.

RESULTS

Frog nerve sartorius muscle preparation : CP (150-600 *μg/ml*) produced a dose related blockade of indirectly evoked twitches (Fig. 1 ; Table I) with no change in direct

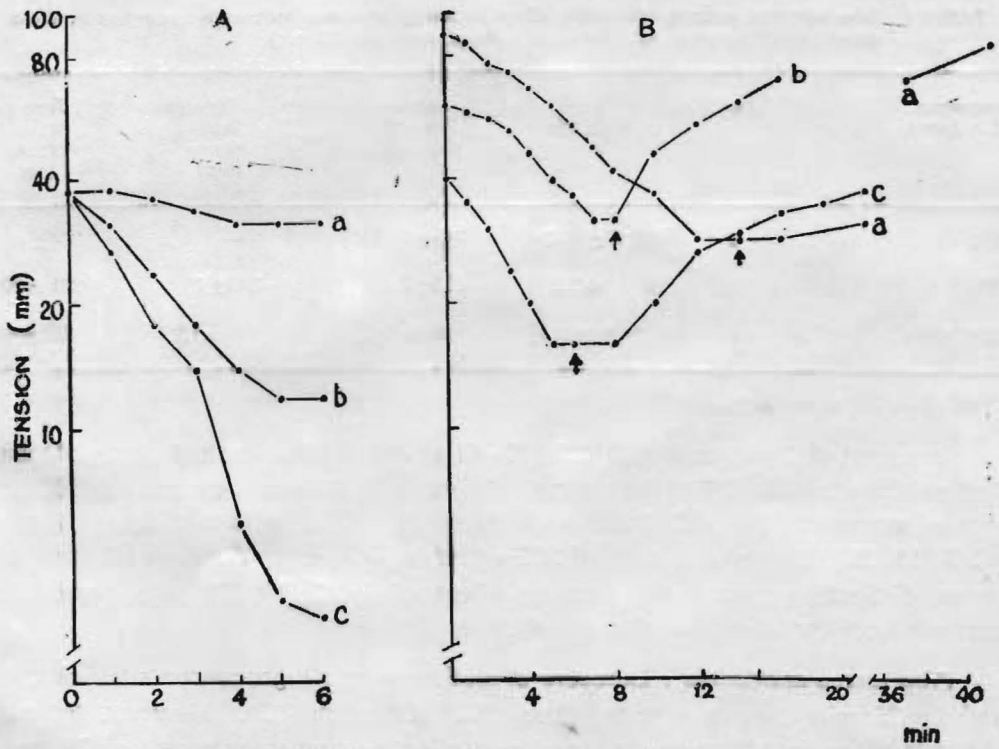

Fig. 1 : Frog sartorius muscle. Centrophenoxine-induced blockade of indirectly evoked twitches plotted on semi-logarithmic coordinates (vertical axis : 56 mm=1 g tension). A : Effect of centrophenoxine alone 150, 300 and 600 *μg/ml* in a, b and c, respectively). B : Effect of centrophenoxine (300 *μg/ml*) and its alleviation by choline chloride (200 *μg/ml*, a), potassium chloride (330 *μg/ml*, b) and adrenaline (1 *μg/ml*, c) added at the arrow.

muscle response. The effect was quickly reversed by a wash. No tachyphylaxis was seen upto 6 consecutive exposures. The time course of decline of twitch tension was exponential after a brief latency period. so that the rate of onset of blockade could be estimated from $T\frac{1}{2}$ (from time-effect plots; see Methods) just like maximal blockade resulting after a given dose.

It was found possible to reverse the blocking effect of CP (300 $\mu g/ml$) by addition of adrenaline (1 $\mu g/ml$, n=9), potassium chloride (330 $\mu g/ml$, n=3) or choline chloride (200 $\mu g/ml$, n=3) to the bath. These concentrations of the antagonists *per se* had no effect on twitch responses. It could be confirmed (13) that choline (500 $\mu g/ml$ or more) itself led to a transient neuromuscular blockade. The effect CP and ability of these drugs to alleviate the CP-blockade is illustrated in Fig. 1. The block due to CP could also be reversed in 8–14 min by physostigmine (1 $\mu g/ml$). If a preparation was bathed in fluid containing physostigimine for 30 min, CP did produce the usual blockade, which, however, disappeared itself in 6-9 min even if CP was not washed out.

TABLE I : Frog sartorius muscle. Blocking effect of centrophenoxine on twitch response to indirect stimulation (supramiximai shocks, 0.5 msec. once per 10 sec).

Concentration µg/ml in bath)	*n=*	*% blockade*	*Latency period (sec)*	*Time to half decay (sec)*	*Time for maximal blockade (sec)*
150	5	39.67±11.2	80±11.7	–	204±45
300	11	56.86±2.2	62±3.4	240±26.5	285±20
600	5	94.4 ±3.8	43±4	117±1.9	260±48

All values are means ±S.E.M.

In a total of 7 experiments, HC 3 (30-40 $\mu g/ml$) led to a gradual decay of indirectly evoked twitches by 40-70 % of the control in 15 min. If CP was now added (600 $\mu g/ml$) it clearly worsened the HC 3-blockade (Fig. 2). Choline is a classical antagonist of HC 3 (11, 12), but even a dose of 200 $\mu g/ml$ (which *per se* does not affect the twitch response) distinctly increased the blocking effect of HC 3 (Fig. 2), an unexpected result which was confirmed repeatedly (n=4).

Frog rectus abdominis : Exposure of recti even to a high concentration of CP (600 $\mu g/ml$) for 30 min did not alter the dose-effect curves for ACh. However, the potency of curare in blocking ACh response was found to be reduced in presence of CP, an effect which was maximal at 160 $\mu g/ml$. Though this was seen in most experiments (5 out of 8) determination of pA_2 values of curare showed that this effect of CP was not statistically significant (pA_2 values before and after CP : 6.16±0.07 and 5.96±0.09, n=8).

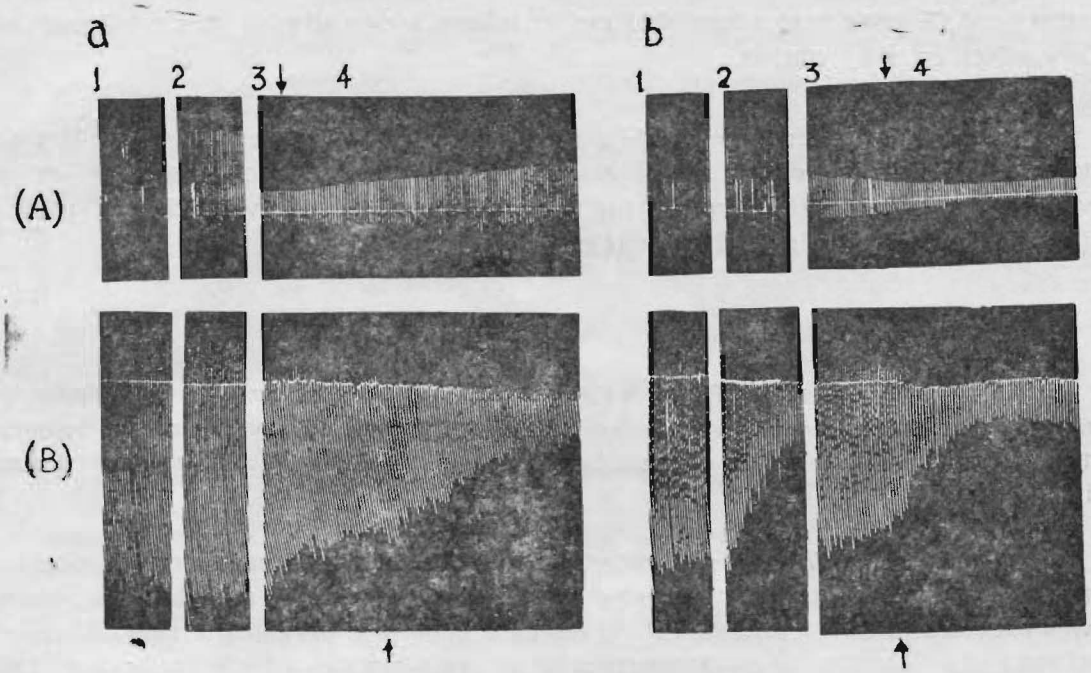

Fig. 2 : (A) Male rat (250 *g*) · urethane anaesthesia. Record of twitch response of gastrocnemius muscle to indirect stimulation. The responses (control : a-1, b-1) were not changed 10 min after iv injection of choline chloride (1 *mg*, a-2) or centrophenoxine (12 *mg*, b-2). Hemicholinum 3 (1 *mg*, slow iv) partially blocked the transmission, effect being maximal after 20 min (a-3, b-3). The blockade was reversed by choline (a-4) but not by centrophenoxine (b-4) given iv (at the arrow).

(B) Sartorius muscle of the frog. Record of indirectly evoked twitches. The response (a-1, b-1) was not altered by choline (200 μ*g/ml*, a-2) but was reduced by centrophenoxine (300 μ*g/ml*, b-2). 15 min after hemicholinium 3 (30 μ*g/ml*) there was a partral blockade (a–3, b-3) which by further increased by choline (a-4) as well as by centrophenoxine (b-4) added at the arrow.

The twitch response of recti subjected to field stimulation was always totally blocked by tubocurarine (0.5 μ*g/ml*) and hence, indicated a totally indirect effect. CP (125 μ*g/ml*) produced 75 to 90% reduction in twitch responses in 5 to 7 min. The response was reproducible without tachyphylaxis and could be reversed by washing out the drug or after addition of adrenaline (1 μ*g/ml*, n=3).

The rat phrenic-nerve diaphragm and chick biventer cervicis : Although tubocurarine (0.5 μ*g/ml* could completely block the indirectly induced twitches each time, even high concentrations of CP (600 μ*g/ml*) were totally without effect on direct or indirectly evoked twitch responses of these preparations.

The rat sciatic nerve-gastrocnemius preparation : Tubocurarine (2-4 μ*g*/rat, slow iv) could always reduce the indirectly evoked contractions of the muscle. The blockade

varied from 40-60% of control response and lasted for 25 to 40 min (n=6). On the other hand CP (even upto a dose of 12 *mg*/rat, infused iv over 10 min) was totally without any effect on the twitches.

HC 3 (n=3) was given slowly iv till a dose of 1 *mg*/rat was delivered. This led to a 30 to 60% reduction of twitch response in 20-25 min. The blocking effect of was not altered by CP (6 *mg*/rat, iv) but was always reversed by iv infusion of choline (1 to 1.5 *mg*/rat, iv) in 15-25 min (Fig. 2).

DISCUSSION

Even high doses of CP did not affect the skeletal neuromuscular transmission in rat as well as in one model of transmission in chick. Unlike the mammalian central neurones the mammalian or avian motor nerves either do not take up CP or they are not responsive to it.

Only the frog *in vitro* preparations showed response of some pharmacological interest to CP. Since the drug is structurally very close to choline, a choline-like activity was expected in CP. Likewise, CP did resemble choline in exhibiting a mild anti-curare effect and in capacity to block transmission in nerve-sartorius muscle preparation (13). Further, both drugs accentuated the HC 3 effect on the latter preparation in present work. Anti-curare effect of CP was not quantitatively impressive and was not further explored. The other two similarities bettween CP and choline need some further consideration.

The effect of choline on the nerve-sartorius preparation was ascribed to decreased sensitivity of motor endplates (see 13) rather than to persistant depolarization. CP is devoid of any such effect since exposure of racti even to high doses of the drug did not alter its sensitivity to ACh, though even smaller doses reduced indirectly evoked contractions of this muscle (field stimulation experiments) and of sartorius muscle. In fact, no proof was obtained to suggest that the drug has a nonspecific effect like membrane stabilization or a specific effect on chlinoceptor or muscle contractility. We therefore presume that CP acts presynaptically and reduced synthesis and/or output of ACh from the motor nerve (see 13) which could lead to a transmission blockade. Our presumption is strengthened by the finding that measures which increase the synthesis/output of ACh, e.g. potassium, adrenaline and choline (3, 14) countered the blocking effect of CP.

In view of the aforesaid, it was of interest to see if HC 3 and CP act silmilarly or interact if co-administered. HC 3 is a classical analytical tool which blocks the neuromuscular transmission in mammals by competing with choline for transport to the intraneuronal sites and by reducing synthesis of ACh (14). A classical test of HC 3-like action,

therefore, is the reversal of blockade by choline. Interestingly, unlike choline, CP could not reverse HC 3 blockade in the rat. This accords with the observation (11) that choline analogues generally do not antagonize HC 3 (unless they promptly release choline). While failure of CP to antagonize HC 3 was thus to be expected even in the frog, two findings were surprising, viz., choline also was ineffective against HC 3 and that CP as well as choline aggrevated HC 3 blockade. Clearly, the effect of HC 3 in frog needs a better understanding. Possibly, choline (itself a blocker of transmission in frog) showed an additive effect with HC 3 in absence of a competition between each other.

On the other hand, choline did antagonize CP and hence seems to compete with it for neuronal uptake. A possible competition between choline and CP was suggested by others also (10). In frog, CP thus seems to act in the manner HC 3 classically acts in mammals.

Since CP has no curarelike effect, its antagonism by physostigmine is more difficult to explain. Recently, physostigmine was shown to reduce the choline uptake by motor nerve endings in rat diaphragm (8) and by cerebral cortex (2) *in vitro*. Since choline and CP seem to share common uptake mechanism in frog motor nerve elements, physostigmine may also reduce CP uptake and hence its blocking activity.

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