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Curare protection against succinylcholine action on muscle

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IT HAS been reported by Ochs *et al.*[1] that succinylcholine applied to frog sartorius muscle greatly reduces twitch heights in response to single direct stimuli. Further, it was shown that curare pretreatment blocks the action of succinylcholine, and it was also found that succinylcholine produces a generalized decrease in resting membrane potentials that is not restricted to end-plate regions. Katz and Miledi[2] have criticized this work and have suggested that the decrease in twitch height observed by Ochs *et al.* may have been due to inadequate direct stimulation or to conduction block at depolarized junctions. Katz and Miledi found that the primary depolarizing action of succinylcholine is restricted to the motor end plates. Further, they found that the effect of succinylcholine on the twitch response is only temporary and follows approximately the same time course as block of the tibial conducted spike when the frog sartorius muscle is directly stimulated on the pelvic end. This indicates that the decreased contractile response is due to a conduction block at the temporarily depolarized end plates. Katz and Miledi did not try curare pretreatment because they felt that there was no general muscle action of succinylcholine to antagonize. The following experiments were undertaken to test possible antagonism by curare of the temporary succinylcholine action.

Frog sartorius muscles, from winter *Rana pipiens*, were stretched to 120% of their 'zero tension' length. The muscles were completely immersed in phosphate-buffered Ringer's solution (with or without curare and/or succinylcholine, pH 7·2, temperature 20° ± 0·4°) and stimulated by two silver electrodes in the solution, one electrode being near each end of the muscle. A square-wave stimulus of 1 msec and 135 V was used. Most of the muscle fibers must have been receiving a direct stimulus, since added curare had only a slight effect on twitch strength. Single isometric twitches were recorded by means of strain gages mounted on a brass torsion bar and a standard amplifying and recording system.

The results (Fig. 1) confirm the finding of Ochs *et al*[1]. that curare protects against the decrease in contractile strength produced by succinylcholine. However, these authors presented data only for the first 10 or 15 min after exposure to succinylcholine. Had they continued, they probably would have seen the recovery observed here. This indicates that the interpretation of Katz and Miledi was probably correct and is consistent with the finding in cat gracilis muscle that curare pretreatment abolishes the end-plate region block to propagation of a directly excited muscle action potential, such temporary block being produced by decamethonium or acetylcholine.[3] It is interesting to note

that curare has also been reported to decrease the neuromuscular blocking effect of succinylcholine.[4]

The generalized depolarizing action of succinylcholine when applied to frog muscles, found by Ochs *et al.*, remains unexplained, but it is of interest that others have reported a similar finding for succinylcholine and decamethonium.[5] Perhaps the discrepancies between these findings and those of Katz and Miledi and other investigators referred to by them, are the result of differences in methods.

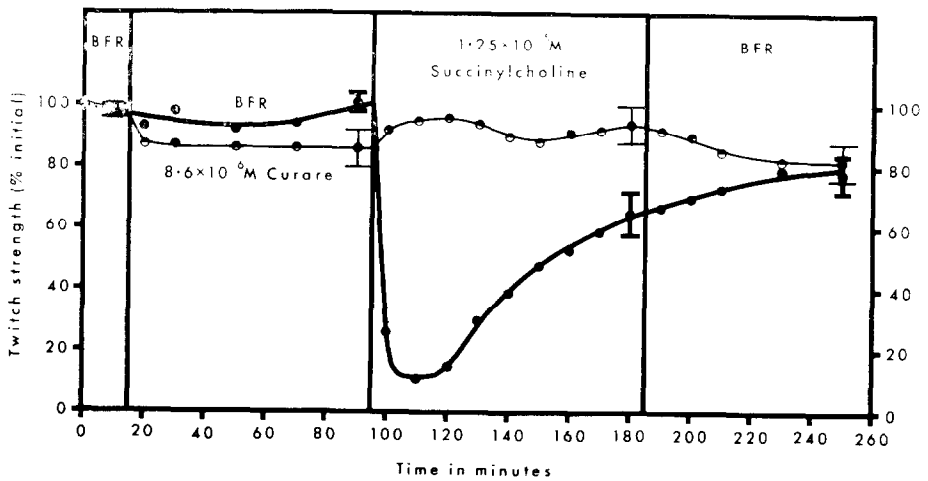

FIG. 1. Vertical bars represent changes in the bathing media as designated. BFR = buffered frog Ringer's. The succinylcholine solution of the curare (*d*-tubocurarine)-pretreated muscles also contained curare. The 'flags' shown are ± the standard error (S.D./$\sqrt{n}$). The dots represent the means taken from eight (BFR control group) or four (curare group) muscles.

The present work is consistent with the view that succinylcholine and analogs initially depolarize the motor end plates and that after about 15 min the motor end plates begin to repolarize, as reported by Thesleff[6] and others. Probably curare prevents this temporary depolarization by combining more firmly with receptor sites than does succinylcholine. Evidence for a competition of this sort between curare and acetylcholine has been obtained by measurements of changes in end-plate potentials in response to ionophoretically applied drugs.[7]

It would appear then, that motor end plates depolarized by succinylcholine block the conduction of action potentials,[2, 3] thereby reducing the twitch response to direct stimuli, and that the temporary end-plate depolarization is prevented by curare pretreatment, thus preventing the decrease in twitch strength.

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Effect of pyridoxine deficiency on intestinal histaminase in the rat

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HISTAMINASE is one of the major enzymes responsible for inactivation of histamine in the rat,[1, 2] and the small intestine has high enzyme activity.[2, 3] It is generally considered to be a flavoprotein,[2, 4–6] with pyridoxal as the coenzyme.[7, 8] It might therefore be supposed that pyridoxine-deficient animals would show diminished histaminase activity, which would be restored by administering pyridoxine to the intact animal or by adding pyridoxal phosphate to isolated enzyme systems *in vitro*. There is a preliminary report in the literature suggesting that the latter occurs.[7] Furthermore, since it has been reported recently[6] that a highly purified histaminase enzyme from hog kidney contains flavine-adenine-dinucleotide (FAD) and pyridoxal as prosthetic groups, the action of both these substances on the histaminase activity of intestine from pyridoxine-deficient rats was considered of interest.

EXPERIMENTAL

Pyridoxine-deficient diet. Four pairs of litter-mate, male, Sprague-Dawley rats (45–60 g) were used. One rat of each pair was fed a pyridoxine-deficient diet *ad libitum*, while its litter mate served as the control, being pair-fed an identical diet with the addition of 80 μg pyridoxine HCl daily. The diet used was based on that described previously,[9] with the exception that pyridoxine HCl was omitted for the deficient rats, and thiamine HCl included. Both deficient and control animals received 1% phthalylsuphathiazole (Thalazole, May and Baker) in their diets, which were given for 30–43 days.

Preparation of histaminase. The histaminase activity of the small intestine, excluding the duodenum, was measured in four paired experiments, with tissues from one pyridoxine-deficient rat and one pair-fed, litter-mate control, both tissues being subjected to identical procedures. Histaminase determination was made as follows. After death by stunning and exsanguination, the small intestine was removed, washed through with 0·9% saline and dried gently between filter papers. The tissue was finely minced with scissors and an extract prepared by grinding with sand in a mortar with 4 vol. saline. The extract was centrifuged (at room temperature) at 8,500 rev/min (7,500 *g*) for 20 min. The resulting supernatant was used as a crude source of histaminase.

Estimation of histaminase activity. Histaminase activity was estimated in 50-ml Erlenmeyer flasks, with the following incubation mixture: histaminase extract, 2·0 ml (equivalent to 0·5 g tissue); 0·2 M sodium phosphate buffer, pH 7·4, 3·0 ml; 0·9% saline, 1·0 ml. In experiments 1–3 where additions were made, pyridoxal-5′-phosphate (100 μg) or FAD (50 μg), or both, were dissolved in 0·2 ml of the 1·0-ml saline and added to aliquots of the histaminase extract, prepared from the same animal. Incubation of the mixture was for 1 hr at 37° in pure oxygen in a metabolic shaker. This was preceded by a 10-min preincubation period before 200 μg of the substrate, histamine acid phosphate, was added. The reaction was stopped by addition of sufficient 2 N HCl to reduce the pH to approximately 5, and by boiling for 1 min. The incubation mixture was then cooled, filtered, and made up to 100 ml. An aliquot was assayed for histamine on the atropinized guinea-pig ileum in an automatic apparatus.

Histaminase activity is expressed as micrograms histamine base inactivated per gram tissue per hour.

RESULTS AND DISCUSSION

Before considering the effect of pyridoxine deficiency on intestinal histaminase, it is necessary to offer some explanation for the rather large reduction in activity that occurerd in the control group with increasing time on the diet (Table 1) This reduction probably reflected the diminution in overall food