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Inhibition of cholinergic response by taurine in frog isolated skeletal muscle

ANDERS LEHMANN*, ANDERS HAMBERGER, *Institute of Neurobiology* and Department of Zoophysiology, University of Göteborg, P.O. Box 33031, S-400 33, Göteborg, Sweden*

Carbamylcholine-stimulated contractions of isolated frog gastrocnemius muscle were inhibited in a non-competitive fashion by 5–25 mM taurine. Taurine had no effect on the resting length of the muscle. Caffeine-induced contractures were unaffected by taurine which indicates that the sarcoplasmic reticulum is not an important site of action for taurine. A possible functional role for taurine in skeletal muscle is discussed.

The β-amino acid taurine is present at high concentration in skeletal muscle (Jacobsen & Smith 1968). The possible physiological role of taurine in muscle is unknown, but it is feasible that calcium fluxes could be regulated or modified by taurine in accordance with the situation in other tissues (Chovan et al 1980; Read et al 1980; Franconi et al 1982a, b). Taurine affects various electrophysiological and biochemical parameters of the sarcolemma and of the sarcoplasmic reticulum (SR). In-vitro, taurine hyperpolarizes rat and frog muscle fibres and decreases the duration of the action potential (Gruener et al 1975). Also the calcium-sequestering capacity of SR, isolated in the presence of taurine, is enhanced (Huxtable & Bressler 1973). In myotonic patients it forestalls the potassium-induced muscular hyperexcitability (Durelli & Mutani 1979).

Reports are sparse on the effect of taurine on skeletal muscle contractility. In-vivo studies have revealed a taurine-induced depression of the twitch-response after nerve stimulation. The lack of inhibition after direct stimulation of the muscle was interpreted as an effect of taurine on neuromuscular transmission (Baskin & Dagirmanjian 1973). The effect of low concentrations has been investigated on muscular contractility in-vitro by Raghu et al (1980), but the results are not conclusive in the context of our question. The present report concerns the effects of taurine on carbamylcholine- and caffeine-induced contractures of the frog isolated gastrocnemius muscle.

Methods

Frogs (*Rana temporaria*) were obtained from Xenopus Ltd, Surrey, U.K., and kept in plastic containers at +4 °C. After decapitation of the animal, the gastrocnemius muscle was dissected and mounted in an organ bath, containing amphibian Krebs-Ringer phosphate buffer with the following composition (mM): NaCl 111, 2; KCl 1,9; $CaCl_2$ 1,1; $Mg SO_4$ 1,6; $Na H_2PO_4$ 0,45; $Na_2 HPO_4$ 2,6 and glucose 5,5 (pH 7,4). The solution was gassed with 100% O_2 and the temperature was 20 ± 1 °C. One end of the muscle was fixed to a platinum-rod (parallel to the longitudinal axis of the muscle) and the other was connected to an isotonic contraction transducer (Gould model 33-03-9811). Fluctuations in muscular length were recorded by a Servogor recorder. After a stable baseline was achieved, a concentration-response curve for carbamylcholine (Sigma) was determined by addition of the drug to the bath in a cumulative way. The carbamylcholine concentration was raised immediately when the equilibrium response had been obtained. The preparation was then rinsed with Krebs-Ringer solution and when the initial resting length had been restored, taurine (Fluka AG) was added to the medium at a concentration of 5 or 25 mM.

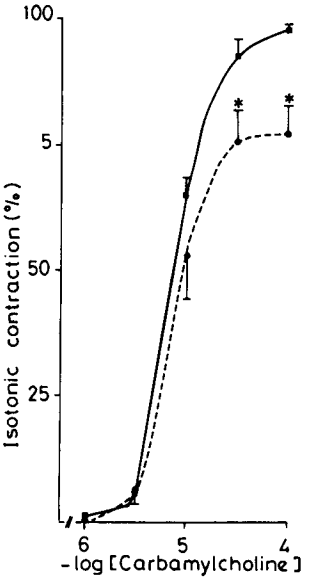

FIG. 1. The concentration-response relation for carbamylcholine (logarithm for molar concentration, solid line) and carbamylcholine in combination with taurine (5 mM, interrupted line). Carbamylcholine was given cumulatively and when taurine was employed, the muscle was preincubated in it for 30 min and exposed to it during the entire experiment. * Denotes a significance level at 0·025 (Wilcoxon matched-pairs, signed-ranks test).

* Correspondence.

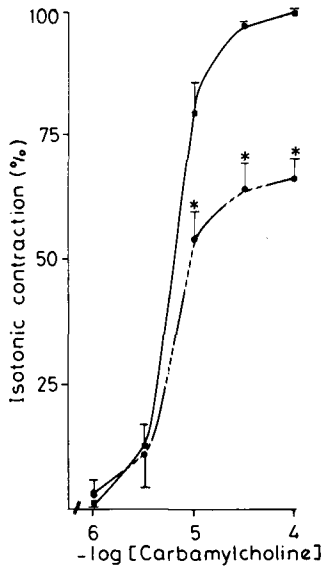

FIG. 2. The concentration-response relation for carbamylcholine (logarithm for molar concentration, solid line) and carbamylcholine in combination with taurine (25 mM, interrupted line). Carbamylcholine was given cumulatively and when taurine was employed, the muscle was preincubated in it for 30 min and exposed to it during the entire experiment. * Denotes a significance level at 0·025 (Wilcoxon matched-pairs, signed-ranks test).

After 30 min, a new concentration-response graph was recorded for carbamylcholine. In control experiments, the same procedure was followed either without amino acid addition, or in the presence of 25 mM leucine (Sigma). Treatment of isolated muscle preparations with high concentrations of caffeine is detrimental to the muscle fibres probably due to a rapid increment in free sacrcoplasmic calcium ions (Endo 1977; Duncan & Smith 1978). Hence, in the caffeine experiments, each muscle could not serve as its own control. The above procedure was followed and caffeine (Sigma) was either administered alone, or in combination with 5 or 25 mM taurine in which case the muscle was preincubated for 30 min. For statistical evaluation, the Wilcoxon matched-pairs, signed-ranks test was used.

Results

Concentration-response graphs for carbamylcholine are shown in Figs 1 and 2. A measurable contracture was seen with 1 μM carbamylcholine and at 100 μM, the shortening was maximal. Higher concentration resulted in a decline of the contracture. Taurine did not affect the resting length, but the maximal reponse was depressed by 23% (5 mM) and 34% (25 mM taurine). In control experiments, the second concentration-response curve was identical to the first and 25 mM leucine had no effect. 1 to 10 mM caffeine elicited isotonic contractures which were unaffected by 5 or 25 mM taurine.

Discussion

The experimental design has permitted us to pin-point taurine's site of action more accurately than has been previously possible. A presynaptic effect of taurine cannot be excluded, but the data indicate a postsynaptic site of action in agreement with Huxtable & Bressler (1973) and Gruener et al (1975). The results do not support the in-vivo studies of Baskin & Dagirmanjian (1973). The latter authors elicited contraction by direct electrical stimulation of the cat anterior tibialis muscle which makes comparisons with the present data uncertain. Our results do not rule out a taurine-inhibited release of acetylcholine, as observed in brain slices (Muramatsu et al 1978). The recent discovery that a key enzyme in taurine biosynthesis, cysteine sulphinic acid decarboxylase, co-exists with choline acetyltransferase in the motor neuron terminal (Chan-Palay et al 1982) is in support of a presynaptic role for taurine.

It has been noted that taurine exerts an antimuscarinic effect in sympathetic ganglia under certain conditions (Hilton 1977). A direct antinicotinic action of taurine can be excluded in the present experiment as the antagonism by taurine as of the unsurmountable type. Therefore it is conceivable that the taurine-mediated inhibition could be attributed to a decrease in sarcolemmal excitability.

Caffeine is internalized within muscle fibres (Bianchi 1962) and releases calcium from the SR (Endo 1977). Impairment of the calcium sequestering capacity of the SR appears to be an additional component of the caffeine effect (Weber & Herz 1968). Taurine did not influence the caffeine-stimulated contractures which constitutes evidence against the SR being an important site of action of taurine. It cannot be excluded, however, that a stimulatory effect on calcium uptake in the SR was exerted by taurine, by analogy with results on isolated SR from skeletal muscle (Huxtable & Bressler 1973). The substantial release of calcium and subsequent rapid increase in free sarcoplasmic calcium could easily mask this effect.

The physiological significance of the present findings is unclear. Although the taurine concentrations used are high, they are of the same magnitude as those in skeletal muscle (Jacobsen & Smith 1968). Therefore, it is not impossible that secretion of taurine from the muscle fibres to the extra-cellular fluid could modulate the contractile performance of the muscle. The elevation of endogenous levels of taurine in dystrophic chicken muscle (Wilson et al 1965) is an indirect indication of a possible functional role for taurine in skeletal muscle. In summary, the results show that taurine depresses skeletal mucle contractures in-vivo, probably via an effect on the sarcolemma.

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Increased brain uptake of morphine in the presence of the antihistamine tripelennamine

N. L. VADLAMANI, R. B. PONTANI, A. L. MISRA*, *New York State Division of Substance Abuse, Research Laboratory, 80 Hanson Place, Brooklyn, N.Y. 11217, and State University of New York, Department of Psychiatry, Downstate Medical Center, Brooklyn, N.Y. 11203, U.S.A.*

Disposition of [6 ^{3}H (N)]morphine in plasma, brain and liver of rats was studied 15 min after intravenous injection of either a 2 mg kg^{-1} dose of morphine or a combination of the same dose of morphine with a 6 mg kg^{-1} dose of tripelennamine. The concentrations of morphine in brain and the brain to plasma morphine ratios in animals receiving the combination of drugs concurrently were significantly higher than those in the control morphine group. No significant differences were seen in the morphine or morphine metabolite concentrations in plasma and liver or liver to plasma morphine concentration ratios in the 2 groups. Data suggest that pharmacokinetic factors play a role in the potentiation of opiate effects by antihistamine on concurrent i.v. administration of the two drugs.

Intravenous abuse of combinations of the antihistamine tripelennamine (Pyribenzamine) with pentazocine (Bhargava 1981; Poklis 1982) or paregoric and morphine (Wendt et al 1964; Burton et al 1965; Szwed 1970) recognized under the street names of 'T's and Blues' and 'Blue Velvet' respectively among heroin addicts in major metropolitan areas of midwestern U.S.A., have received increasing attention in the last five years. These combinations reportedly produce a heroin-like 'rush' sensation lasting for 5 to 10 min, followed by a feeling of well-being over the next 4 h. The addicts carefully establish a correct ratio for these drugs by titrating themselves to get the sought-after 'high', while avoiding a seizure. The abuse of such combinations is potentially dangerous and sometimes fatal. A non-lethal dose of tripelennamine in combination with pentazocine resulted in a significant decrease in the LD50 of pentazocine from 116 to 60 mg kg^{-1} i.p. in mice (Waller et al 1980). Concomitant s.c. administration of tripelennamine with pentazocine or morphine shifted the dose-response curve of the opiate to the left and enhanced the duration of the antinociceptive effect of narcotics (Tagashira et al 1982) and morphine-like discriminative stimulus effects of pentazocine (Shannon & Su 1982). At present, the rationale underlying the use of combinations of an antihistamine and opiate and the enhancement of narcotic effects is not clear. This investigation was undertaken to determine any possible role of altered distribution factors in the potentiation of opiate effects on concurrent i.v. administration of tripelennamine and morphine.

* Correspondence.

Methods

Samples of drugs. Tripelennamine hydrochloride was a gift from Ciba-Geigy Corp., Summit, N.J [6-^{3}H (N)]-morphine, specific activity 9·84 Ci m mol^{-1} was obtained commercially from New England Nuclear Corp. Boston, Mass. The ethanol solution of the