Action of Nicotinic Stimulant Agents on Rabbit Skeletal Muscle I. Nicotine and Acetylcholine. (20682)

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Langley and Dickinson(1) preventing nicotine tremors by section of the motor nerves in *anesthetized mammals* gave evidence of their central origin; this locus of action was confirmed by Amantea(2) and Galamini(3), who noted motor stimulation after cortical application of nicotine, and by Longo and Bovet(4), who recorded brain waves similar to *grand mal* seizures in curarized rabbits receiving repeatedly high doses of nicotine. Nevertheless, the peripheral stimulant effect of nicotine(5,6) and acetylcholine(7) on mammalian skeletal muscle is also unequivocally established.

Our recent observations of the blockade by various neuromuscular agents(8), and ether (9) of nicotine-induced tremors in unanesthetized rabbits prompted a reinvestigation of the problem in *unanesthetized unmedicated animals.*

Materials and methods. The rabbit was selected as the most suitable animal for study in the unanesthetized state. Eighteen male albino New Zealand rabbits, weighing between 1.5 and 2.5 kg were used in 30 assays. One hindleg was denervated by section of the sciatic nerve, while the contralateral leg in the same animal was used as control. All animals were tested 30 minutes after denervation. The survivors, however, were tested again 3, 5, 8, and 10 days after denervation. Both early and late tests were considered. For testing muscle tremors, the same procedure as described by Longo and Bovet(10) was used, with the addition that independent movements of both hind legs were recorded simultaneously. For recording individual isotonic muscle contractions, rabbits were prepared as follows. The Achilles tendon was exposed, severed just proximal to the calcaneum, and securely tied by a nylon thread to an isotonic muscle lever. The leg was immobilized by clamps holding a rod drilled through the lower part of the femur.* For experiments with ether the sectioned tibial nerve, exposed just below the knee, was stimulated by means of shielded electrodes. Stimulation was provided by an electronic square wave "Electrodyne" at a voltage of 200 and a frequency of 0.5 pulse per second. Nicotine tartrate, 0.5% aqueous solution, was injected at a dose of one mg/kg into the marginal ear vein. Acetylcholine (10 μg/kg) was injected by the same route 10 minutes after intravenous injection of 10 mg/kg atropine sulfate and 5 minutes after one mg/kg physostigmine salicylate. Ethyl ether (for anesthesia) was given by inhalation in anesthetic concentrations.

Results. Effects of nicotine upon denervated rabbit muscle. No significant difference was noted in the results whether experiments were conducted either immediately after or some days after the section of the nerve. When tremors induced by a small dose of nicotine in the denervated hindleg were compared with those recorded in the contralateral normal leg of the unanesthetized rabbit, a positive response was noted in both legs in 6 out of 6 experiments; of these the amplitude and duration were either the same (3 cases) or greater in the denervated hindleg (Fig. 1). In 19 experiments, isotonic muscle contractions induced by nicotine were compared in both hindlegs. In 14 animals the response was the same in both hind legs. In 4 the response was smaller in the denervated leg (Fig. 2), and in one it was even greater. A longer induction period was noted in the denervated leg than in the normal one.

Effect of acetylcholine in the atropinized-eserinized rabbit. In 5 experiments, acetylcholine induced contractions of about the same magnitude in the denervated and the normal contralateral leg muscle.

Effects of ether on nerve muscle preparation of the rabbit. Light anesthesia induced

* We are indebted to Dr. Walter Riker for demonstration of this method.

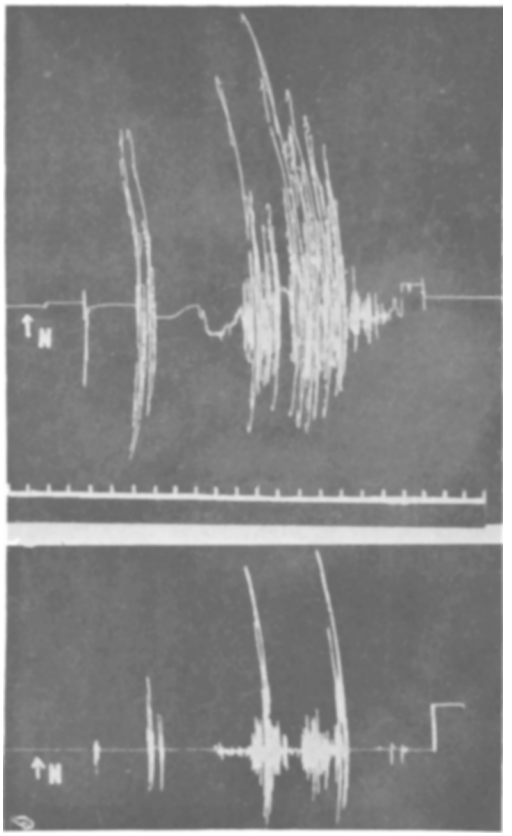

FIG. 1. Comparative tremors induced by nicotine in acutely denervated hind leg (upper tracing) and contralateral normal leg (lower tracing) of an unanesthetized rabbit. Time in 5 sec. N: Nicotine tartrate 1 mg/kg inj. into ear marginal vein.

by ether inhalation caused a temporary excitation followed by a decrease of approximately 80% in the response to sciatic stimulation. This effect was reversible and disappeared gradually after removal of the anesthetic.

Discussion. Our data, which give evidence that in the unanesthetized rabbit nicotine induced tremors and muscle contractions are not prevented by acute or chronic section of the motor nerves, do not agree with the results obtained in *anesthetized mammals* by Langley and Dickinson(1) who state, "After section of the nerves which run to any part of the body, we have not seen either a movement or twitching take place in this part on injecting nicotin. . . . It will be remembered that in the frog nicotin has a slight peripheral action and thereby causes a faint fibrillar twitching of the muscles." Undoubtedly, their results were conditioned by the use of ether which was omitted in our experiments. It seems reasonable to suggest that ether may interfere with the effect of nicotine at the motor endplate of the neuromuscular junction. This suggestion is in accord with earlier data which gives evidence of the neuromuscular blocking effect of ether, either alone in mammals (11,12), and in man(13), or in enhancing d-tubocurarine in animals(14-16), and in man(17). Such a mechanism for the action of ether would account for our earlier observation of the ether blockade of nicotine induced tremors(9). Besides the central stimulant effect of nicotine, which was recently reemphasized by Longo and Bovet(4), one must conclude that nicotine exerts a separate peripheral action. This fact should be considered in the interpretation of the blockade of nicotine-induced tremors in screening anti-Parkinsonism drugs.

Summary. In the *unanesthetized rabbit,* tremors of the hind legs and muscle contractions of the gastrocnemius muscle induced by intravenous doses of nicotine tartrate or acetylcholine are not prevented by acute or chronic section of the sciatic nerve. It is concluded that nicotine exerts, in addition to a central action, a separate and distinct

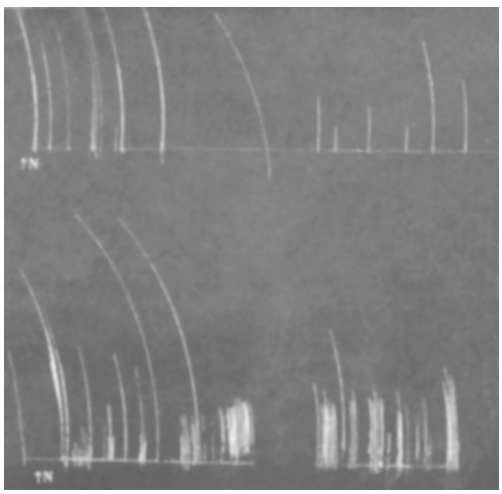

FIG. 2. Comparative isotonic muscle contractions induced by nicotine in acutely denervated gastrocnemius (upper tracing) and contralateral normal muscle (lower tracing). N: Nicotine tartrate 1 mg/kg inj. into ear marginal vein.

peripheral effect on mammalian striated muscle.

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Relative Inability of Heparin to Prolong Clotting Time of Oxalated and Resin-Treated Blood and Plasma.*† (20683)

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In the course of studies on the anticoagulant activity of heparin it was noticed that this drug failed to prolong the clotting time of recalcified, oxalated or resin-treated blood and plasma. In view of the possible importance of this phenomenon as a source of error in the evaluation of the anticoagulant activity of heparin, it was decided to investigate the matter further.

Materials and methods. A) *Collection of blood and plasma in various anticoagulants.* Blood was collected from healthy donors in Silicone-coated glass syringes through Arquad 2-C‡ coated needles. It was transferred to Silicone-coated test tubes and made incoagulable by the addition of one of the following reagents: (a) sodium or potassium oxalate, 0.1 M; (b) sodium citrate, 0.2 M; (c) Sequestrene-Na_2§ 1% in 0.7% NaCl, all in the volume of one ml to 9 ml of blood. In other experiments, whole blood was decalcified by passage through a column of cation-exchange resin (2.5 g of resin to 10 ml of blood) with the technic described by one of us(1). Dowex-50‖ and IR-100¶ in the sodium cycle were used in this work. In all cases plasma was separated by centrifugation of the whole blood at 2000 rpm for 10 minutes. B) *Addition of heparin.* Saline solution containing various amounts of heparin was added, in the

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‡ Dicoco-dimethyl-ammonium chloride; available from Armour Co., Chicago, Ill.

§ Disodium ethylendiamine-tetracetate-dihydrate; available from Alrose Chemical Co., Providence, R. I.

‖ Available from Dow Chemical Co., Midland, Mich.

¶ Available from Rohm and Haas Co., Philadelphia, Pa.,