trione-3-arylhydrazone (0.005 mole) dissolved in an alcohol–acetic acid mixture. The resultant solution was boiled under reflux for several hours and then cooled. The reaction mixture was diluted with water, and the crystals which separated were collected and purified by recrystallization from ethanol. Characteristics of N^1-(4-methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles are listed in Table II.

N^1-Hippuryl-3,5-dimethyl-4-arylazopyrazoles—These compounds were obtained from hippurylhydrazine (0.005 mole) and 3-arylhydrazono-2,3,4-pentanetrione (0.005 mole) by the same procedure as was adapted for N^1-(4-methoxyphenylsulfinyl)-3,5-dimethyl-4-arylazopyrazoles. The yields and physical constants of these pyrazoles are listed in Table III.

N^1-Hippuryl-3-methyl-4-arylazo-5-phenylpyrazoles—Treatment of 2-arylhydrazono-1-phenyl-1,2,3-butanetriones with hippurylhydrazine under conditions similar to those used in other cases gave the pyrazole derivatives listed in Table IV.

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Classification of Nicotine Block at Neuromuscular Junction

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Abstract □ The effect of nicotine on the electrical threshold of the neuromuscular junction in a rat sciatic-gastrocnemius preparation was studied and compared with the effects of a true curarizing agent and those of a pseudocurarizing agent on the threshold of the like structure of the same preparation. Low doses of both nicotine and succinylcholine chloride caused a decrease in the predrug electrical threshold level of the neuromuscular junction, while high doses of these drugs caused an elevation of the predrug threshold level. Both low and high doses of dimethyl tubocurarine chloride, on the other hand, caused an elevation in this threshold level. Eserine salicylate enhanced the early blockades caused by nicotine and succinylcholine chloride but opposed the late blockades caused by these same drugs. Eserine salicylate opposed both the early and the late stages of a neuromuscular blockade brought about by dimethyl tubocurarine chloride. Nicotine and succinylcholine chloride induced a spastic paralysis in chicks, whereas dimethyl tubocurarine chloride induced a flaccid paralysis in other chicks of the same age and weight. On the basis of these studies, nicotine is classified as a neuromuscular blocking agent of the pseudocurare type which does not exert its effect through acetylcholine release.

Keyphrases □ Nicotine—effect on electrical threshold, neuromuscular junction, rat sciatic-gastrocnemius preparation, classified as neuromuscular blocking agent of pseudocurare type □ Neuromuscular blockage—nicotine effects studied, compared to other agents, classified, using rat sciatic-gastrocnemius preparation

Nicotine is thought to bring about a stimulation of ganglionic and related receptors, causing a depolarization of the postsynaptic membrane and a transient response. If the dose is adequate, it is thought to block somehow these same receptors as a result of, first, a prolonged depolarization and, later, a stabilized polarization phase.

The block of nicotine at the ganglion was considered by Pelikan (1) to result from an ability to prevent the release of acetylcholine at this site and not from its ability to compete with acetylcholine for the site receptors. On the other hand, Lundberg and Thesleff (2) recognized the ability on the part of nicotine *per se* to stimulate at the ganglionic receptor sites, but they stated that there is no complete correlation between the ganglion blocking action of nicotine and its prolonged ganglion cell depolarization because the blocking action outlasts the depolarization. The work of Paton and Perry (3) supported the view that the ganglionic block of nicotine has a second phase which occurs after the membrane becomes repolarized.

Langley (4), in 1909, discovered an antagonism by curare of nicotine's effect at the neural region of the amphibian skeletal muscle fiber. Thesleff (5), in 1955, found that the neuromuscular blockades of nicotine, succinylcholine, decamethonium, and acetylcholine develop during the depolarization of the muscle sole plate and continue after the transmembrane potential of this structure is spontaneously restored, without removal of the depolarizing agent. He concluded that the neuromuscular blocking action of these drugs is the result of a decrease in the sensitivity of the endplate to the neuronal transmitter.

Beani and Bianchi (6, 7), on the basis of their work in the guinea pig phrenic nerve-diaphragm preparation, classified nicotine as a stimulatory neuromuscular blocking agent. However, they agreed with Thesleff that its competitive block of cholinergic receptors at the

Table I—Effect of Low and High Doses of Neuromuscular Blocking Agents on Electrical Thresholds of Rat Sciatic-Gastrocnemius Preparations

	Change in Threshold[a], %		
	Nicotine	Decamethonium	Succinylcholine
Low dose	−39 ± 2[b]	+7166 ± 3244	−35 ± 14
High dose	+189 ± 38[c]	+7347 ± 4639	+255 ± 73

[a] Calculated as the difference between thresholds before and 15 min. after administration of blocking agent, expressed as percent of threshold before first injection. [b] A minus (−) sign indicates that threshold decreased after the drug. [c] A plus (+) sign indicates that threshold increased after the drug. Each value represents the mean ±*SEM* of three to five experiments.

neuromuscular junction is its major inhibitory mechanism with respect to skeletal muscle contraction.

The study reported here represents an attempt to determine, on the basis of the relative effectiveness of antidotal antagonism of nicotine, succinylcholine, and curare blocks, the proper classification of the neuromuscular blockade of nicotine. It also represents an attempt to determine whether the effect of nicotine at the neuromuscular junction is a direct or an indirect effect, the latter achieved through the inhibition of acetylcholinesterase.

EXPERIMENTAL

Adult female rats, weighing between 175 and 250 g., were anesthetized with a solution containing 50 mg. chloralose and 500 mg. urethan/ml. at a dose of 1.5 ml./kg. body weight, administered intraperitoneally. The trachea was exposed and cannulated with a 5.08-cm. (2-in.) piece of tubing (PE 205). Through a sidearm of the tubing, the animal was allowed to respire unassisted until such time as this should be impossible; then the sidearm was closed and the respiratory pump was turned on.

The left external jugular vein was exposed and cannulated with tubing (PE 50) attached at its opposite end to a saline-filled syringe. All drugs were administered through the sidearm of this tubing and were flushed into the animal with 0.2 ml. of saline. The dorsal muscles of the right hind leg were exposed and the gastrocnemius was isolated, a ligature being placed around the Achilles tendon. The tendon was then cut distal to the ligature, and the muscle was attached by way of a pulley to a lever weighted with 5 g. Contraction of the muscle exerted an upward pull on the short (attached) end of the lever, moving the opposite end downward on the fulcrum. Affixed to the end moving down was a short piece of wire. If the muscle contraction were adequate, this wire made contact with a brass plate 2 mm. beneath, closing the circuit in which the lever was incorporated and lighting a bulb. Extreme care was taken during the preparation to ensure an uninterrupted blood supply to the gastrocnemius. At the end of each individual determination, the muscle was cut in half. If no observable bleeding occurred, results of the determination were discarded.

The sciatic nerve was exposed close to its point of emergence from the vertebral column. After the nerve was sectioned, its peripheral end was secured to shielded electrodes (HA 305), and single shocks from a stimulator (AEL 104 A) were applied to the nerve at the rate of one every 10 sec. Only the intensity of these shocks was varied. A liquid petrolatum coating kept the nerve from drying out. Special care was taken to immobilize the hind leg so that gastrocnemius contractions would move only the attached muscle lever. The muscle was kept moist and warm with normal saline (37–38°) dripping from a buret positioned above.

Threshold voltage was specified as that which was just adequate when applied to the muscle through the sciatic nerve to elicit a contraction response sufficient to move the tip of the attached lever downward a distance of 2 mm. At the end of this excursion, the lever tip made contact with the brass plate, closing the circuit and lighting the bulb to indicate attainment of the threshold response. Threshold voltage was determined by serial increases in the intensity of single induction shocks applied to the preparation.

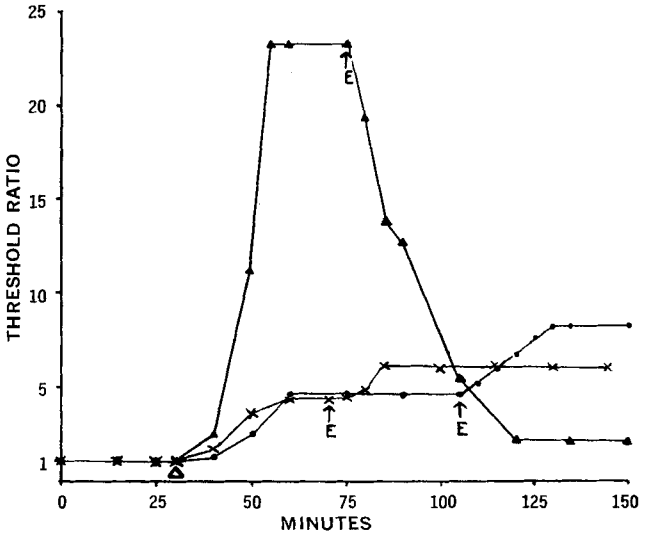

Figure 1—*Effect of eserine salicylate (E) on the change produced in the electrical threshold of the rat sciatic-gastrocnemius preparation by nicotine (·—·), dimethyl tubocurarine chloride (——), and succinylcholine chloride (×—×). The open triangle (△) indicates the first injection of the blocking agent. Each line represents the results of one experiment. Doses of eserine were the same. The threshold ratio is the electrical threshold (in volts) at any given time divided by the electrical threshold at zero time.*

Threshold voltage was determined every 5 min. Drugs were not administered unless the same threshold voltage was obtained for six successive determinations (30 min.). Low and high cumulative doses were employed.

At preestablished "low" doses, nicotine, dimethyl tubocurarine chloride, and succinylcholine chloride (0.100, 0.010, and 0.001%, respectively) showed some neuromuscular blocking activity but did not affect respiration. "High" doses of these agents (1.000, 0.100, or 0.010%, respectively) were sufficient to inhibit spontaneous respiration while exerting marked neuromuscular blocking activity. All solutions were given intravenously in an injection volume of 1.0 ml./kg. Three to five animals were used for each experimental condition tested.

A dose of blocking agent was administered every 5 min. until an inhibition of neuromuscular transmission was evidenced in an elevation in threshold voltage. The new threshold voltage was then determined every 15 min. thereafter for a period of not less than 2 hr. All doses of blocking agent were submaximal; that is, the inhibition of transmission could be overcome with an electrical stimulus of sufficient intensity.

In the first portion of the study related to nicotine's blocking mechanism, the rectus abdominis muscle of the frog was suspended in a 50-ml. bath chamber containing modified Ringer solution (100 ml. Ringer diluted to 140 ml. with water) through which oxygen was bubbled. The responses of rectus muscles during a series of 90-sec. contacts with nicotine (10^{-3} mm.) (8) after exposure to the acetylcholinesterase of horse serum for 15 min. were compared with

Table II—Effect of Eserine Salicylate on Early and Late Phases of Neuromuscular Blockades Produced by Nicotine, Dimethyl Tubocurarine, and Succinylcholine

Phase of Blockade	Change in Threshold[a], %		
	Nicotine	Dimethyl Tubocurarine	Succinylcholine
Early (<120 min.)	+35 ± 22[b]	−79 ± 3	+28 ± 7
Late (>120 min.)	−62 ± 1[c]	−78 ± 7	−56 ± 1

[a] Calculated as the difference between thresholds before and 15 min. after administration of drug, expressed as percent of threshold before first injection. [b] A plus (+) sign indicates enhancement of the neuromuscular blockade. [c] A minus (−) sign indicates antagonism of the neuromuscular blockade. Each value represents the mean ±*SEM* of three to five experiments.

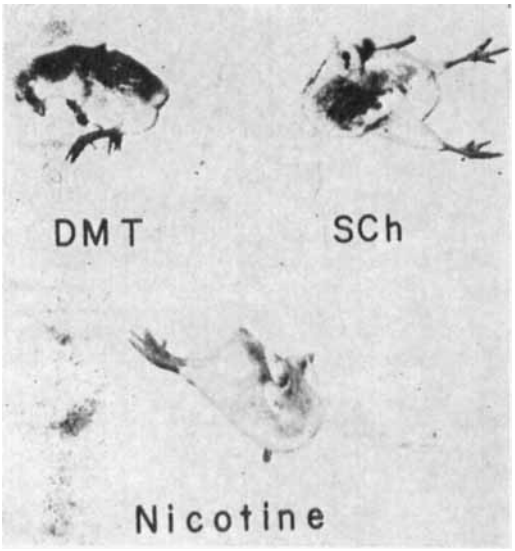

Figure 2—*Effect of intravenous injection of dimethyl tubocurarine chloride (DMT), succinylcholine chloride (SCh), and nicotine on 4–7-day-old chicks.*

the responses prior to exposure. These responses to nicotine were then compared with the responses to eserine following exposure of the latter to acetylcholinesterase. In the second portion of the study, the rabbit duodenal strip was suspended in oxygenated (95% O_2, 5% CO_2) Ringer solution (0.1% dextrose added) in the inner chamber of a constant-temperature bath kept at approximately 37°. The effect of eserine added to the chamber was recorded, and the strip was washed with a fresh dextrose–Ringer solution and allowed to return to its previous tone. Then the effect of an ensuing 12–15-min. suspension of the strip in regular (dextrose-free) Ringer was recorded. At the conclusion of this period, the effect of eserine added to the chamber was recorded during a 3-min. exposure and compared with that of nicotine added to the chamber a short time later.

RESULTS AND DISCUSSION

Low doses of nicotine and succinylcholine repeatedly diminished the threshold voltage of this rat sciatic-gastrocnemius preparation while high doses repeatedly elevated this threshold voltage. Any effective dose of dimethyl tubocurarine, on the other hand, only elevated threshold voltage (Table I).

In the early phase (within 1.5–2 hr. after injection) of the nicotine and succinylcholine blocks, eserine repeatedly elevated threshold voltage, enhancing these blocks (Fig. 1); it repeatedly lowered the threshold voltage of the late phase (more than 2 hr. after injection) of the nicotine and succinylcholine blocks, opposing these blocks.

In both early (Fig. 1) and late phases of the dimethyl tubocurarine block, eserine repeatedly lowered the threshold voltage (Table II).

According to Buttle and Zaimis (9), the intravenous injection of depolarizing blockers into young chicks, 4–7 days old, causes a rigid extension of the head and legs. Intravenous injection of polarizing blockers, on the other hand, causes a flaccid paralysis of the head and legs. Nicotine and succinylcholine injected intravenously into such chicks produced a spastic extensor paralysis of the neck and legs, whereas dimethyl tubocurarine produced a flaccid paralysis of the neck and legs (Fig. 2).

A number of investigators believe that the effect of nicotine is achieved both centrally and peripherally as a result of acetylcholine release. Accordingly, an attempt was made to determine whether the neuromuscular blocking action of nicotine is dependent upon the release and/or maintenance of acetylcholine. The availability of acetylcholine was diminished to the point of no response to eserine (in this study) in the frog rectus abdominis muscle by exposure of the tissue to acetylcholinesterase (of horse serum). In the isolated rabbit ileum, acetylcholine was depleted after immersion in a dextrose-free Ringer solution as determined by the absence of response to eserine (10). In both preparations, nicotine exerted a strong stimulant effect shortly after the absence of response to eserine.

According to the results presented, nicotine, unlike the so-called pseudocurares, does not turn its attention from acetylcholinesterase receptors to the cholinergic receptors of the neuromuscular junction during its skeletal muscle blocking action. Rather, nicotine seems to exert a direct effect throughout, first bringing about a depolarization of the muscle sole plate through union with the cholinergic receptors of the neuromuscular junction, then somehow stabilizing these same receptors (after repolarization of the muscle fiber membrane). Nicotine's depolarization of the muscle sole plate through union with its cholinergic receptors is responsible initially for its threshold-lowering effect at the neuromuscular junction and shortly thereafter for the first or depolarizing phase of its blocking action. The subsequent stabilization of the same muscle sole plate receptors is responsible for the second or polarizing phase of its blocking action.

Nicotine, although its effect pattern simulates that of the pseudocurare agents, gives evidence of belonging in a class of neuromuscular blocking agents by itself, by virtue of the fact it achieves that pattern throughout by acting directly on the receptors of the neuromuscular junction.

SUMMARY

1. Like succinylcholine, nicotine first diminishes and later elevates threshold voltage of the neuromuscular junction. Dimethyl tubocurarine only elevates threshold voltage of the neuromuscular junction.

2. During the early phase of nicotine's threshold elevation, eserine contributes to the elevation; during the late phase of nicotine's threshold elevation, eserine antagonizes the elevation. Eserine has the same qualitative, if not quantitative, effect on early and late phases of succinylcholine threshold elevation. On the other hand, eserine antagonizes both the early and late phases of dimethyl tubocurarine's threshold elevation. Nicotine and succinylcholine produce a spastic paralysis of the head and legs of the young chick; dimethyl tubocurarine produces a flaccid paralysis of these members.

3. The action of nicotine at the neuromuscular junction appears to be direct rather than indirect through the release and/or maintenance of acetylcholine.

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