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Cardiac Vagolytic Action of Some Neuromuscular Blockers[1]

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PUNNEN, S., E. R. GONZALEZ, A. J. KRIEGER AND H. N. SAPRU. *Cardiac vagolytic action of some neuromuscular blockers.* PHARMACOL BIOCHEM BEHAV **20**(1) 85–89, 1984.—Cardiac vagolytic effect of four commonly used neuromuscular blockers, (viz. D-tubocurarine, decamethonium, pancuronium and gallamine) was compared in midcollicular decerebrate rats. The intravenous doses of neuromuscular blockers used (d-tubocurarine: 0.1 mg/kg; decamethonium: 2 mg/kg; pancuronium: 0.1 mg/kg; gallamine: 20 mg/kg) were sufficient to produce the paralysis of respiratory muscles. Bradycardia was induced by electrical stimulation of the vagus or by injecting dimethyl-phenyl-piperazinium (DMPP; a ganglionic stimulant). It was observed that d-tubocurarine and decamethonium were devoid of cardiac vagolytic action. On the other hand, pancuronium and gallamine inhibited significantly the bradycardia induced by electrical stimulation of the vagus or injection of DMPP; gallamine was found to have greater vagolytic action. The pressor responses to DMPP were not attenuated by pancuronium and gallamine indicating that in the dose administered, these agents did not block the ganglia. Bradycardia induced by the administration of acetylcholine in the left atrium was also attenuated by pancuronium and gallamine suggesting that the drugs produce cardiac vagolytic action by acting on the post-synaptic cholinergic receptors of the heart.

Acetylcholine Bradycardia DMPP Neuromuscular blockers Vagolytic

DURING investigations on cardiovascular function, it is often necessary to immobilize and artificially ventilate experimental animals in order to prevent cardiovascular changes secondary to blood gas alterations [2]. Some of the neuromuscular blockers used to immobilize the experimental animals (e.g., gallamine triethiodide) are known to selectively block the cardiac vagus nerve [7]. Such a blockade of the cardiac vagus nerve complicates the interpretation of cardiac function in these immobilized preparations. The present investigation was undertaken to compare the cardiac vagolytic action of four commonly used neuromuscular blockers viz. gallamine, pancuronium, decamethonium and d-tubocurarine to determine if one or more of them would show no cardiac vagolytic action in doses sufficient to produce a neuromuscular blockade. It is not known whether blockade of the cardiac vagus produced by some of these neuromuscular blockers (e.g., gallamine) is at the parasympathetic ganglion or a peripheral site [7]. An attempt was made, therefore, to determine the site of blockade of these neuromuscular blockers on the cardiac vagus nerve.

METHOD

Animal Model

A midcollicular decerebrate preparation of male Wistar rats, weighing 400–450 g, was used in each experiment. The details of the decerebration procedure are described elsewhere [5]. In all experiments, both of the femoral arteries were cannulated; one for withdrawing blood samples for blood gas analyses and the other for monitoring blood pressure via a pressure transducer (Statham, P 23 Db). Heart rate was monitored by a tachograph (Grass 7P4) which was triggered by blood pressure pulses. Mean blood pressure, was computed electronically from the systolic-diastolic blood pressure. Pulsatile blood pressure, mean blood pressure and heart rate were continuously recorded on a polygraph (Grass 7D). One of the femoral veins was cannulated for making injections. The rectal temperature was continuously monitored and maintained at 37±0.5°C by a temperature controller (Bailey instruments, BAT-8). The animal was fixed in a supine position and ventilated artificially using a rodent respirator (Harvard, 680). The rate, tidal volume and end-expiratory pressure of the respirator were adjusted so that the blood gases and pH remained within normal limits [5]. Blood gas analyses were performed on four femoral arterial blood samples (0.6 ml each) using a blood gas analyzer (Instrumentation Labs, IL micro 13); frequent withdrawal of blood samples was avoided to prevent excessive blood loss. A total of 50 rats was used in this study; the number of animals used in each series of experiments is indicated in the following sections.

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Electrical Stimulation of the Vagus

Twenty decerebrate rats were used in this series of experiments (5 rats for each neuromuscular blocker). The vagus nerves were exposed under an operation microscope (see diagram in [4]) and sectioned 1 mm caudal to the nodose ganglion. The caudal end of the right or left sectioned vagus nerve was placed on a bipolar platinum-iridium electrode which was connected to a stimulator (Grass S88) and immersed in a pool of warm (37°C) paraffin oil. Bradycardia was produced by electrically stimulating the vagus nerve for 15–30 sec. The stimulus parameters were as follows: frequency 1, 5, 10, 15 and 25 pulses per sec (PPS), duration 3 msec and intensity supra-maximal (4–6 volts).

Ganglionic Stimulation with DMPP

Twenty decerebrate rats were used in this series of experiments (5 rats for each neuromuscular blocker). Dimethylphenyl-piperazinium (DMPP) is known to stimulate autonomic (para-sympathetic as well as sympathetic) ganglia [7]. A dose of 200 μg/kg IV of DMPP was selected because this dose was found to produce significant bradycardia due to para-sympathetic ganglion stimulation [6]. Changes in blood pressure as well as heart rate induced by DMPP were measured before and after the administration of each neuromuscular blocker in separate series of experiments. These experiments were done to determine the site of vagolytic action (para-sympathetic ganglia or peripheral site) of the neuromuscular blockers.

Effect of Neuromuscular Blockers on the Heart Rate Changes Induced by Exogenous Acetylcholine

Ten decerebrate rats were used in this series of experiments; 5 for each of the two drugs (gallamine and pancuronium) used. The rats were anesthetized and prepared for cardiovascular measurements and electrical stimulation of the vagus as described above. A premeasured cannula (PE 50) was introduced into the left atrium via the right common carotid artery; the position of the cannula tip was ascertained by the contour of the blood pressure pulses and by postmortem examination. Acetylcholine (1 μg/kg) injected through this cannula reached coronary circulation immediately and induced bradycardia. Subsequently, bradycardia was induced by the stimulation of the vagus. Neuromuscular blockers (gallamine and pancuronium) then were injected intravenously and heart rate changes in response to exogenous acetylcholine and electrical stimulation of the vagus were observed again. These experiments were carried out to determine the site of cardiac vagal inhibitory action (pre- or post-synaptic) of the neuromuscular blockers [3].

Preparation and Administration of Drugs

The solution of each drug was freshly prepared in physiological saline (pH 7.4). The following drugs were used in this study: gallamine triethiodide (FLAXEDIL; American Cyanamid, NY; 20 mg/kg, i.e., 8.97×10^{-6} moles/kg, IV), Pancuronium bromide (PAVULON; Organon, NJ; 0.1 mg/kg, i.e., 5.46×10^{-8} moles/kg, IV), decamethonium bromide (Sigma, MO; 2 mg/kg, i.e., 1.91×10^{-6} moles/kg, IV), and dimethylphenyl-piperazinium-iodide (Sigma, MO; 200 μg/kg, IV), d-tubocurarine (0.1 mg/kg, i.e., 5.87×10^{-8} moles/kg; IV, Sigma, MO), acetylcholine (1 μg/kg, IV; Sigma, MO).

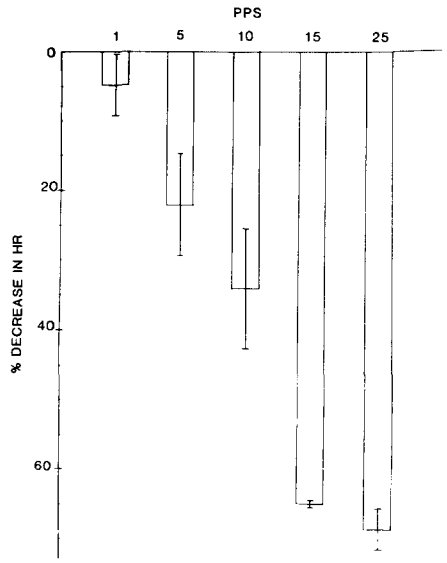

FIG. 1. Bar-graph showing heart rate responses to variable frequencies of stimulation of the vagus (n=20). The magnitude of bradycardia (expressed as % decrease in heart rate) increased as the stimulus frequency (pulses per sec; PPS) was increased. The duration of the stimulus was 3 msec and the intensity was supramaximal (4–6 volts).

Statistical Analysis

Since each animal served as its own control, a paired *t*-test was used to determine significance of difference between means [1]. Differences were considered significant at $p<0.05$.

RESULTS

Effect of Neuromuscular Blockers on the Bradycardia Induced by Electrical Stimulation of the Vagus

The control values for the systolic, diastolic (S/D), and mean blood pressure (MBP) and heart rate (HR) were as follows: 144±5 mmHg, 106±5 mmHg, 118±7 mmHg, 437±17 beats/min, respectively. The electrical stimulation of the vagus produced instant bradycardia; the percentage decreases in heart rate at different stimulus frequencies (with stimulus intensity supramaximal 4–6 volts, and duration 3 msec) are shown in Fig. 1. At least 3 control responses of bradycardia were obtained before a neuromuscular blocker was administered to the animal. Preliminary experiments showed that the following intravenous doses of the neuromuscular blockers were sufficient to stop spontaneous respiration for about 30 min: d-tubocurarine chloride (0.1 mg/kg), decamethonium bromide (2 mg/kg), pancuronium bromide (0.1 mg/kg) and gallamine triethiodide (20 mg/kg). Figure 2A shows a typical tracing from one experiment indicating that d-tubocurarine (0.1–0.2 mg/kg) had no significant effect on bradycardia induced by the electrical stimulation of the vagus. Figure 3A shows graphically that d-tubocurarine produced no significant change in the bradycardia induced by the stimulation of vagus in five such experiments. Similarly, decamethonium did not alter vagally mediated bradycardia (Figs. 2B and 3B). On the other hand, the bradycardia induced by the electrical stimulation of the vagus was smaller (Fig. 2C) after the administration of pancuronium bromide (0.1 mg/kg IV); the percentage reduction

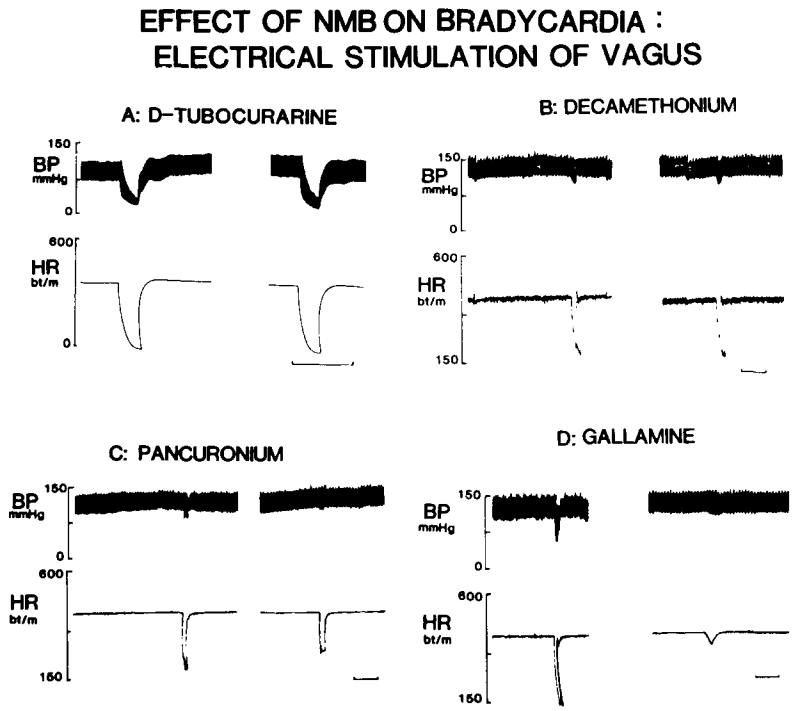

FIG. 2. Tracings of typical experiments showing the effect of neuromuscular blockers on the bradycardia induced by the electrical stimulation of the vagus. In each panel, top trace: pulsatile blood pressure (BP, mmHg); bottom trace: heart rate (beats/min). Time scale, shown (bottom right corner) in each panel, is 1 min. In each panel, tracings of BP and HR before and after the drug are shown on the left and right, respectively. Note that d-tubocurarine (A) and decamethonium (B) did not attenuate the bradycardia induced by the electrical stimulation of the vagus while pancuronium (C) and gallamine (D) produced significant attenuation of the bradycardia. Only one drug was used in one animal.

of heart rate following vagal stimulation was 51.8±3.6% before, and 23.2±2.9% after the administration of pancuronium (Fig. 3C). These differences were statistically significant ($p<0.001$). Gallamine triethiodide (20 mg/kg IV) produced even greater attenuation of bradycardia induced by vagal stimulation (Fig. 2D). The percentage reduction of heart rate was 52.1±2.9% before and 5.7±2.6% after the administration of gallamine (Fig. 3D). These differences were statistically significant ($p<0.001$).

Effect of Neuromuscular Blockers on the Ganglionic Stimulation Produced by Dimethyl-Phenyl-Piperazinium (DMPP)

Previous experiments in this laboratory [6] showed that DMPP (200 μg/kg, IV) produced significant rise in blood pressure with concomitant bradycardia. Decrease in heart rate was not of reflex origin because similar responses were obtained in preparations in which the aortic and carotid sinus nerves were selectively sectioned. DMPP elicited bradycardia by stimulation of para-sympathetic ganglia associated with the cardiac vagus nerve. The polygraph tracings shown in Fig. 4 (A and B) indicate that d-tubocurarine and decamethonium did not attenuate DMPP-induced bradycardia or pressor response. On the other hand, pancuronium and gallamine attenuated the bradycardia, but not the pressor response induced by DMPP (Fig. 4 C and D). The values of DMPP-induced changes in heart rate and blood pressure before and after the administration of the four neuromuscular

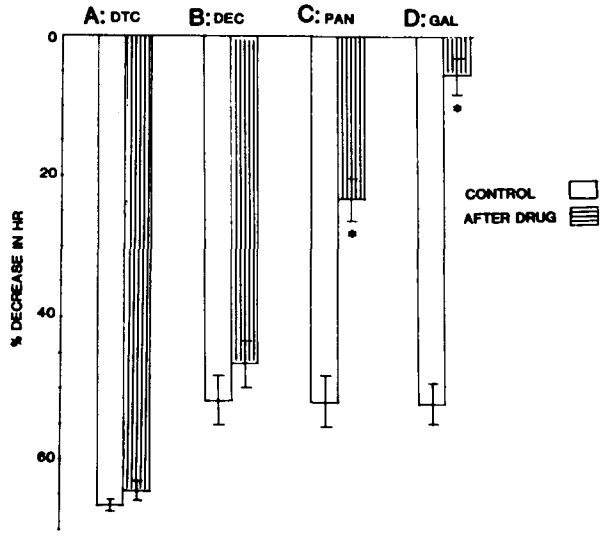

FIG. 3. Bar-graph comparing the vagolytic action of various neuromuscular blockers (n=20; 5 rats for each neuromuscular blocker). Bradycardia was induced by the electrical stimulation of vagus. A: d-tubocurarine (DTC); B: decamethonium (DEC); C: pancuronium (PAN); D: gallamine (GAL). In each pair of bars: blank bar represents bradycardia before drug and hatched bar represents the same parameter after the drug. Asterisk indicates that the values are significantly different from the respective control ($p<0.001$). Values of each bar represent mean±S.E.

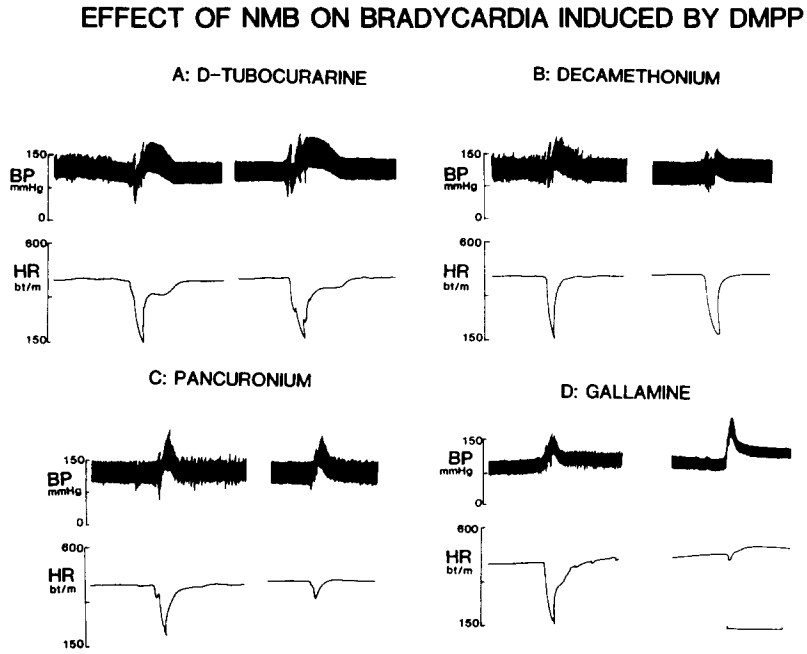

FIG. 4. Tracings of typical experiments showing the effect of neuromuscular blockers on the bradycardia induced by DMPP. Dimethyl-phenyl-piperazinium (200 μg/kg, IV) produced pressor response and bradycardia; the latter response was not reflex in origin because the aortic, carotid sinus and vagus nerves were selectively sectioned. Legends same as in Fig. 2. Note that pancuronium and gallamine attenuated the bradycardia, while d-tubocurarine and decamethonium had no significant effect on this response. None of these agents attenuated the pressor response indicating absence of ganglion blocking properties at the doses used.

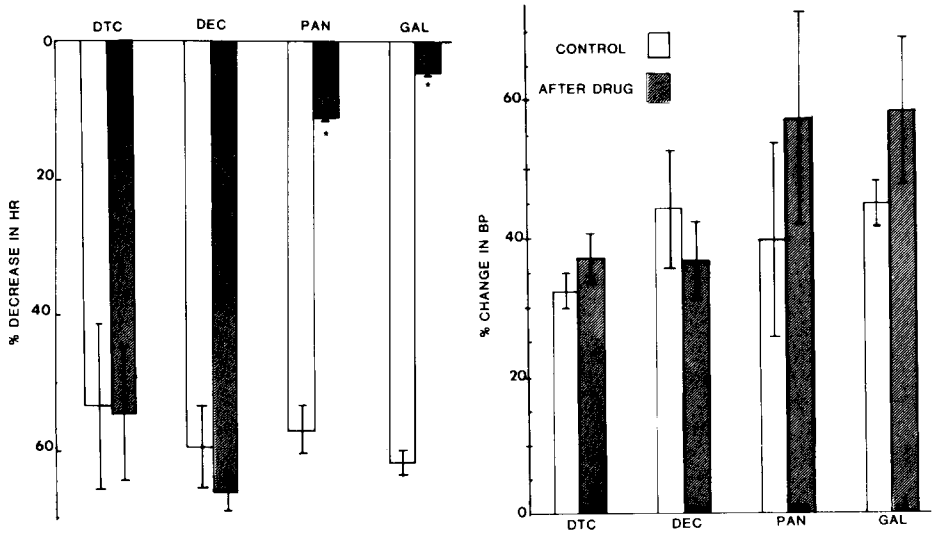

FIG. 5. Bar-graph showing the effect of neuromuscular blockers (NMB) on the heart rate and blood pressure changes induced by DMPP (n=20); 5 for each neuromuscular blocker). Left Panel: Effect of NMB on the DMPP-induced bradycardia. Note the significant attenuation ($^*p<0.001$) of the bradycardic response by pancuronium (PAN) and gallamine (GAL), D-tubocurarine (DTC) and decamethonium (DEC) did not attenuate heart rate (HR) responses. Right Panel: Effect of NMB on the DMPP-induced pressor response. Note no neuromuscular blocker produced attenuation of pressor responses induced by DMPP. Instead, pancuronium (PAN) and gallamine (GAL) exaggerated the pressor responses; however, the exaggeration of the pressor response was not significant.

blockers are shown in Fig. 5; the DMPP-induced bradycardia was significantly smaller ($p<0.001$) after pancuronium and gallamine (left panel), while none of these agents attenuated pressor response to DMPP (right panel).

Effect of Neuromuscular Blockers on the Heart Rate Changes Induced by Exogenous Acetylcholine

Injection of acetylcholine (1 μg/kg, left atrium) and electrical stimulation of the vagus (15 PPS, 4 volts, 3 msec) produced immediate bradycardia. Gallamine (20 mg/kg, IV) and pancuronium (0.1 mg/kg IV) significantly attenuated the bradycardia produced by either acetylcholine or vagal stimulation.

DISCUSSION

The doses of various neuromuscular blockers used in this study were enough to paralyze respiratory muscles in rats for at least 30 min. Detailed dose-response studies were not carried out because the purpose of this investigation was to study the vagolytic action of neuromuscular blockers at the doses used for immobilization. At these doses, cardiac vagolytic action (indicated by attenuation of bradycardia following electrical stimulation of vagus) was exhibited by gallamine and pancuronium; gallamine was much more potent than pancuronium in this action. On the other hand, d-tubocurarine and decamethonium were found to be devoid of significant vagolytic action. The differences in cardiac vagolytic action of these neuromuscular blockers cannot be ascribed to the differences in the molar concentrations of the doses injected. For example, pancuronium and d-tubocurarine were injected in virtually equimolar (5.87×10^{-8} moles/kg) doses; pancuronium showed a significant vagolytic effect at this dose while the same dose of d-tubocurarine was devoid of this action. Bradycardia induced by stimulation of para-sympathetic ganglia using dimethyl-phenyl-piperazinium (DMPP) was also attenuated by gallamine and pancuronium. However, the pressor responses produced by DMPP were not attenuated by these agents; this action indicates that gallamine and pancuronium (in the doses used) did not have ganglion blocking action. Attenuation of bradycardia, induced by electrical stimulation of the vagus or activation of para-sympathetic ganglia by DMPP, must be due to the action of gallamine and pancuronium on post-ganglionic sites associated with the cardiac vagus nerve. DMPP-induced bradycardia was not a reflex response to increase in blood pressure produced by DMPP because in these animals, the aortic nerves, the carotid sinus nerves and the vagus nerves (caudal to the nodose ganglia) were selectively sectioned under an operation microscope. These two agents (gallamine and pancuronium) attenuated or blocked the bradycardia induced by exogenous acetylcholine also. This observation indicates that these neuromuscular blockers block post-synaptic cardiac muscarinic receptors.

In conclusion, the results of this investigation have demonstrated that some neuromuscular blockers (e.g., gallamine and pancuronium) exhibit cardiac vagolytic action. Gallamine is more potent in this action than pancuronium. The site of cardiac vagolytic action of these two agents is post-synaptic cardiac muscarinic receptors. These two agents are not, therefore, suitable for studies in which heart rate changes are to be monitored. On the other hand, d-tubocurarine and decamethonium do not exhibit cardiac vagolytic action; these two neuromuscular blockers are therefore suitable for cardiovascular studies in which animals need to be immobilized and artificially ventilated.

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