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CARDIAC EFFECTS OF VAGAL STIMULATION IN THE ANAESTHETIZED CAT

BY

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Continuous stimulation of the right vagus nerve in anaesthetized animals can produce cardiac asystole, followed by a reinitiation of the heart beat. The latter is commonly referred to as "vagal escape" (Bard, 1956a). Although the mechanism of the escape phenomenon is unresolved, evidence has accumulated which indicates that adrenergic factors may be involved, possibly through a local release of catecholamines by acetylcholine (Campos & Freidman, 1963; Moore, 1967).

To evaluate the local release hypothesis, the effects of beta adrenergic and postganglionic parasympathetic blockade on the chronotropic action of the vagus were determined in this study. In the course of our experiments, interesting data were obtained on the effect of stimulation frequency on the duration of vagally induced cardiac asystole and the intracardiac origin of the ensuing vagal escape. The cat was used in all experiments because the vagus and cervical sympathetic nerves can be easily separated in this species (Liddell & Sherrington, 1929).

METHODS

Cats of either sex, weighing between 2 and 5 kg, were anaesthetized intraperitoneally with α-chloralose, 85 mg/kg, and placed on a heated cat board. A tracheotomy was performed, and the exposed part of the cannula was covered with non-conducting electrical tape. Arterial blood pressure was recorded from the right femoral artery with a cannula leading to a Statham P 23 Db transducer and Sanborn Model 296 recorder. The right femoral vein was cannulated for injection of drug solutions. The Lead II electrocardiogram was recorded from subcutaneous needle electrodes with a Sanborn Model 100 Viso-Cardiette recorder. The left vagosympathetic trunk was isolated and cut. The corresponding right trunk was isolated, the cervical sympathetic section was isolated and severed, and the vagus was tied with suture and crushed central to the tie. Throughout the experiments, the right vagus was kept warm (~37° C) and moist with Locke-Ringer solution.

The vagus was stimulated with bipolar platinum electrodes leading from American Electronics Laboratory stimulus isolation and stimulator units. Supramaximal voltage was determined for each preparation by selecting the voltage required to produce a maximal slowing of the heart rate at a stimulation frequency of 5 c/s.

Experimentation was started 30 min after the supramaximal voltage was established. The right vagus was stimulated supramaximally for a period of 30 sec with square wave pulses of 1 msec duration at frequencies of 5, 10, 20, 40, 60 and 80 c/s. Three initial experiments were performed in which each animal was tested once with each of these frequencies in a random order. No drugs were administered. In a second experimental series, seven animals were tested at frequencies of 5, 10 and 20 c/s, and seven others were tested at 40, 60 and 80 c/s. These frequencies were employed in a random order both before and after intravenous injection of propranolol hydrochloride (200

μg/kg). Four additional animals were treated with atropine sulphate (0.2-1.0 mg/kg) following two control stimulations at 20 c/s. These stimuli were repeated after drug treatment. Physostigmine sulphate (50 μg/kg) was then administered, and the two 20 c/s challenge stimulations were repeated. Stimuli were given at 5 min intervals unless a drug treatment period intervened. All drugs were dissolved in saline and infused intravenously at a rate of 0.5 cm^3/min for 10 min to avoid the fall in blood pressure sometimes seen after rapid injection of atropine and propranolol. Post drug stimuli were begun 10 min after completion of the infusion. The control measurements from all the above experiments were pooled, except where a paired analysis with post drug values was performed.

Asystole was defined as an interval between beats equalling or exceeding 4 sec, and the effect of vagal stimulation was expressed as the total duration of asystole occurring during the 30 sec period of stimulation. To determine the intracardiac site of vagal escape, each of the first three escape beats, following the first initiation of asystole, was classified as either atrial or ventricular in origin on the basis of the presence or absence of a discernible *P* wave in the electrocardiogram. The results obtained from all the animals tested at a given frequency were combined, and the percentage incidence of ventricular escape was determined. The first three beats were arbitrarily chosen for use in our analysis to reflect the immediate intracardiac locus of escape after vagally induced asystole. The term "vagal escape" was defined as the reinitiation of the heart beat during continuous vagal stimulation, after a period of asystole of 4 or more sec.

All drug concentrations refer to the base form of the molecule. Statistical analysis was made by methods described by Snedecor (1956).

RESULTS

The effect of frequency on the total duration of vagally induced asystole, during the 30 sec of stimulation, is shown in Fig. 1. The results show that the relationship between frequency and the total duration of asystole is a bell-shaped curve, the peak of which

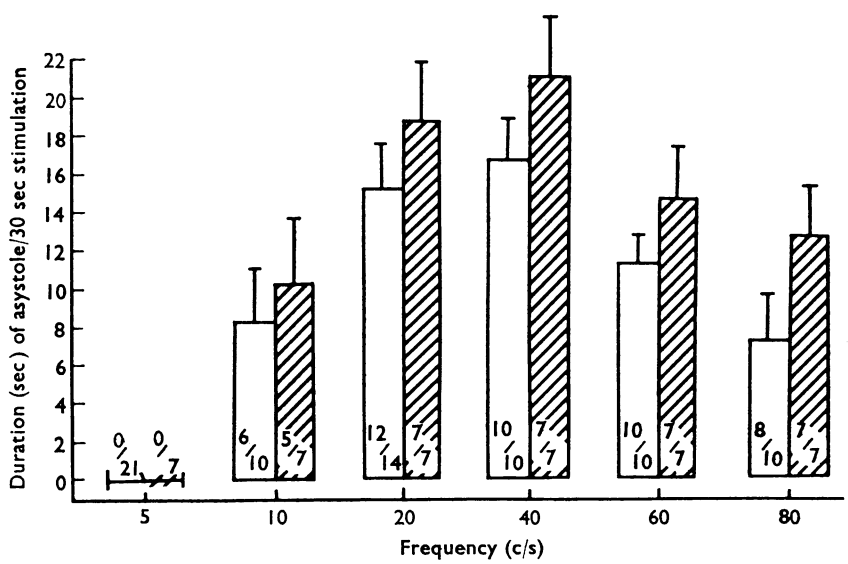

Fig. 1. Relationship between mean duration of asystole and frequency of vagal stimulation in control conditions and after beta adrenergic blockade. Each mean value was calculated on the basis of all animals tested. Fractions within bars indicate incidence of asystole (No. of cats showing asystole/No. of cats tested). Brackets indicate the standard error of the mean. White columns, control ; shaded columns, propranolol (200 μg/kg).

is attained at 20–40 c/s. This interpretation is based on a statistical analysis (Student's *t* test), which showed that the mean duration of asystole was significantly longer ($P<0.05$) at 40 c/s than at 10 or 80 c/s. Treatment with propranolol (200 μg/kg) did not significantly alter the shape of the curve, although when the results were analysed on a paired basis, using each animal as its own control, the average duration of asystole was significantly prolonged at frequencies of 20–80 c/s (Table 1). Evidence that this dose of propranolol produced beta blockade is provided in Table 2, which shows a reduction in resting heart rate after drug administration. Blood pressure was unaffected by treatment with the drug, indicating a lack of severe impairment of myocardial function.

TABLE 1

PROLONGATION OF DURATION OF ASYSTOLE BY PROPRANOLOL (200 μg/kg, INTRAVENOUSLY)

Frequency (c/s)	5	10	20	40	60	80
Mean prolongation (sec)	0	4·5	6·6	5·2	5·5	7·5
S.E.	±0	±2·9	±2·0	±1·3	±2·1	±2·2
N	7	7	7	7	7	7
P	N.S.	N.S.	<0·02	<0·01	<0·05	<0·02

TABLE 2

EFFECT OF PROPRANOLOL (200 μg/kg, INTRAVENOUSLY) ON RESTING HEART RATE (BEATS/MIN) AND BLOOD PRESSURE (mm Hg)

	Heart rate		Systolic pressure		Diastolic pressure	
	Control	Propranolol	Control	Propranolol	Control	Propranolol
Mean	173	144	168	162	123	115
S.E.	±9	±7	±6	±7	±4	±6
N	14	14	14	14	14	14
P	—	<0·02	—	N.S.	—	N.S.

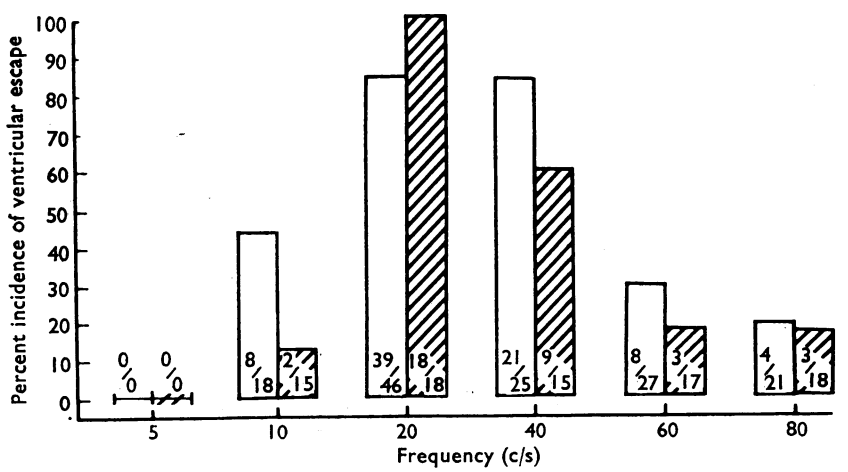

Fig. 2. Relationship between stimulation frequency and incidence of ventricular vagal escape in control conditions (white columns) and after propranolol treatment (shaded columns). Fractions within bars indicate the number of ventricular escape beats obtained, divided by the total number of beats analysed at a given stimulation frequency. In each animal, three beats were evaluated with each frequency at which asystole occurred unless fewer than three beats occurred in the 30 sec stimulation period.

The incidence of ventricular escape was also found to be frequency dependent, and a bell-shaped relationship between these two parameters was observed in both control and propranolol experiments (Fig. 2). Thus, when the vagus was stimulated supramaximally at a frequency of 20 or 40 c/s, sinus dominance was usually lost and ventricular escape occurred ; at frequencies of 10, 60 and 80 c/s, the principal locus of escape was atrial.

A control electrocardiogram tracing obtained in one experiment is shown in Fig. 3. A stimulation frequency of 5 c/s did not produce asystole, although the heart rate was reduced. Stimulation at 10 c/s produced two asystoles of 5.2 and 4.5 sec, respectively. Each asystole was followed by a beat that was atrial in origin, as indicated by the presence of a *P* wave preceding the *QRS* complex. The initial asystole at 20 c/s was 13.1 sec in duration followed by an atrial beat, while a second asystole of 11.1 sec preceded one atrial and a ventricular beat. Stimulation at 40 c/s produced an asystole of

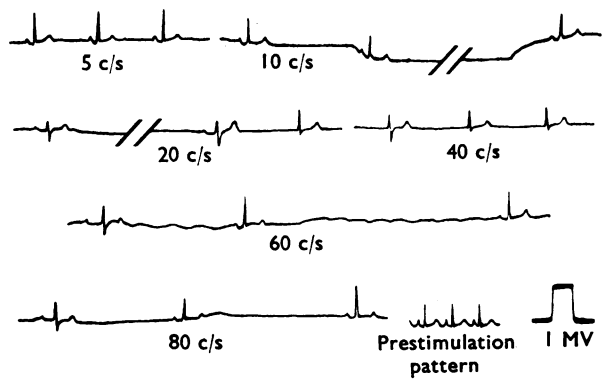

Fig. 3. Control experiment showing first three beats after the first initiation of asystole at frequencies of 5–80 c/s. Recordings shown were traced from original record. Paper speed was 25 mm/sec. Hatched bars indicate break in record during period of asystole.

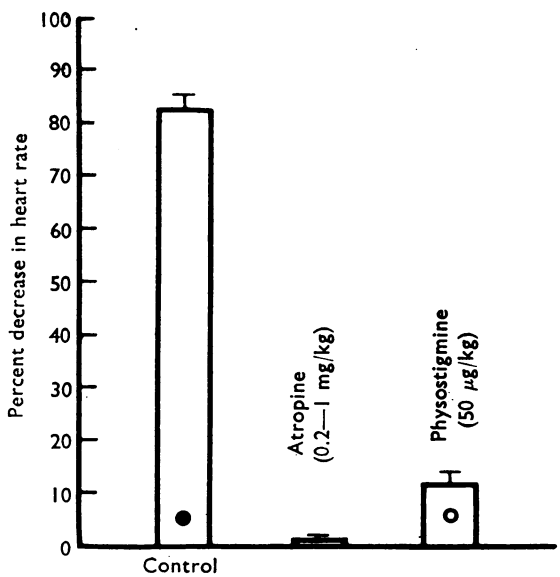

Fig. 4. Effect of atropine and subsequent treatment with physostigmine on the negative chronotropic action of vagal stimulation at 20 c/s. The control bars represent the average chronotropic response to vagal stimulation before atropine administration. Each bar represents the mean of four cats. Brackets indicate standard error of the mean. Statistically significant from zero: (●), $P<0.001$; (○), $P<0.01$.

20.6 sec followed by three ventricular beats, while 60 c/s caused a 12.0 sec asystole followed by three atrial beats. At 80 c/s, three atrial beats were observed after an asystole of 9.0 sec.

The effect of atropine on vagal activity is shown in Fig. 4, which summarizes the results of four experiments ; two of these were done with an atropine dose of 0.2 mg/kg, and two with a dose of 1.0 mg/kg. The results were similar in each experiment, so the results were combined. Atropine completely blocked the negative chronotropic effect of the 20 c/s vagal stimulus. No evidence of a cardioaccelerator action was obtained in any experiment. The subsequent administration of physostigmine (50 μg/kg) enabled the vagus to partially overcome the atropine block.

DISCUSSION

No sympathetic vagal action was unmasked by atropine pretreatment in our experiments. Thus the cat vagus seems to be a pharmacologically pure parasympathetic nerve. These results are in contrast with those reported recently by Randall, Priola & Pace (1967), who found that stimulation of the dog vagosympathetic nerve following atropine resulted in an adrenergically induced cardioacceleration. This dichotomy is possibly caused by the presence of cardioaccelerator fibres in the cervical sympathetic portion of the canine nerve trunk.

The administration of a beta blocking dose of propranolol resulted in a prolongation of the duration of vagally-induced asystole, thus suggesting that adrenergic factors play a part in inducing vagal escape. The most likely adrenergic mechanism to fit this result is that the fall in blood pressure accompanying vagal stimulation reflexly increases sympathetic drive to the heart. This interpretation is compromised somewhat by the fact that, in our experiments, part of the afferent arm of the baroreceptive reflex mechanism was destroyed by interrupting the vagus nerve. This procedure would not, however, destroy the glossopharyngeal nerve, which also is an afferent arm of this reflex (Rushmer, 1961). It is possible that catecholamines were locally released by the acetylcholine released from the vagus, and that the asystole prolonging action of propranolol was caused by an antagonism of these amines. No evidence was obtained for a release of catecholamines by acetylcholine in the atropine experiments, in which the postganglionic parasympathetic cardiac receptors, but not the adrenergic sympathetic receptors, were blocked. These results, however, do not rule out the existence of a peripheral muscarinic receptor for local catecholamine release which would also be blocked by atropine.

It may be argued that the resting heart rate after atropine was so high that further acceleration was impossible, thus " masking " any vagally induced positive chronotropic response. This rate in our experiments (157 ± 8) was, however, substantially below the maximal rate which has been elicited in the cat in similar experimental conditions (Black, Duncan & Shanks, 1965).

The total duration of asystole following vagal stimulation was dependent on the frequency of stimulation in control conditions and after treatment with propranolol. The reason for the decrease in duration at frequencies above 40 c/s in unknown, but may

be related to parasympathetic ganglionic blockade because high frequencies of stimulation through other autonomic ganglia are known to inhibit impulse transmission (Bard, 1956b).

Vagal escape is generally considered to be the result of the unmasking of a ventricular pacemaker while the sinoauricular node is suppressed (Bard, 1956a), but the results obtained in our study suggest that this is generally true in the cat only at frequencies of 20–40 c/s. At frequencies above and below these levels, escape is primarily atrial in origin.

SUMMARY

1. The incidence of ventricular escape following supramaximal stimulation of the right vagus nerve in the anaesthetized cat was found to be dependent on the stimulation frequency. The peak occurrence of ventricular escape was at stimulation frequencies of 20–40 c/s.

2. The total duration of asystole during vagal stimulation was also frequency dependent with the longest durations of asystole occurring at 20–40 c/s.

3. Treatment with propranolol (200 μg/kg) significantly prolonged the total duration of asystole at frequencies of 10–80 c/s.

4. Treatment with atropine (0.2–1.0 mg/kg) completely blocked the negative chronotropic effects of vagal stimulation at 20 c/s. No sympathetic activity was unmasked in these conditions. The subsequent administration of physostigmine (50 μg/kg) resulted in a partial reversal of the atropine block.

REFERENCES

BARD, P. (1956a). *Medical Physiology*, 10th ed., p. 70–71. St. Louis: C. V. Mosby.

BARD, P. (1956b). *Medical Physiology*, 10th ed., p. 996. St. Louis: C. V. Mosby.

BLACK, J. W., DUNCAN, W. A. M. & SHANKS, R. G. (1965). Comparison of some properties of pronethalol and propranolol. *Br. J. Pharmac. Chemother.*, **25**, 577–591.

CAMPOS, H. A. & FREIDMAN, A. H. (1963). The influence of acute sympathetic denervation, reserpine and choline xylyl ether on vagal escape. *J. Physiol., Lond.*, **169**, 249–262.

LIDDELL, E. G. T. & SHERRINGTON, C. (1929). *Mammalian Physiology: A Course of Practical Exercises*, p. 19. London: Humphrey Milford.

MOORE, J. I. (1967). Effect of cocaine on vagal escape in the open-chest dog. *Proc. Soc. exp. Biol. Med.*, **124**, 163–167.

RANDALL, W. C., PRIOLA, D. V. & PACE, J. B. (1967). Responses of individual cardiac chambers to stimulation of the cervical vagosympathetic trunk in atropinized dogs. *Circulation Res.*, **20**, 534–544.

RUSHMER, R. F. (1961). *Cardiovascular Dynamics*, p. 151. Philadelphia: W. B. Saunders.

SNEDECOR, G. W. (1956). *Statistical Methods*, 5th ed., pp. 45–47. Ames: Iowa State College Press.