

## THE INOTROPIC EFFECT ON THE HEART OF STIMULATING THE VAGUS IN THE DOG, DUCK AND TOAD

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### SUMMARY

1. The chronotropic and inotropic effects of stimulating the vagus on the hearts of the dog, duck and toad were studied.
2. The maximum rate of rise of pressure in the left ventricle ( $dP/dt$  max) measured at a constant heart rate and mean aortic pressure was used as an index of the inotropic changes.
3. The sensitivity of  $dP/dt$  max as an index of inotropic changes brought about by stimulating the vagus was established in the toad where a 49% reduction in heart rate was associated with a 30% reduction in  $dP/dt$  max.
4. In the dog stimulation of the vagus resulted in a reduction in heart rate of 38% and only a small reduction in  $dP/dt$  max of 6%.
5. Results similar to those found in the dog were obtained in the duck where the reduction in heart rate of 44% was associated with reduction in  $dP/dt$  max of only 3%.
6. It is concluded that the vagus has only a small and negligible negative inotropic effect on the ventricles of the dog and duck.

### INTRODUCTION

Stimulation of the parasympathetic nerves to the heart is known to result in a decrease in heart rate but the existence of a negative inotropic effect on the ventricles is controversial. The results of earlier investigations both for and against an inotropic effect on the ventricles have been reviewed by MacWilliam (1930). More recent investigations have also resulted in contradictory results, e.g. Sarnoff, Brockman, Gilmore, Linden & Mitchell (1960) who claimed that the vagus had no inotropic effect on the ventricles and De Geest, Levy, Zeiske & Lipman (1965) who claimed that the vagus had a significant negative inotropic effect.

The purpose of this present investigation was to determine the effects of stimulating the vagus on the left ventricle using the maximum rate of

rise of pressure in the ventricle ( $dP/dt$  max) as an index of inotropic changes. It is known that  $dP/dt$  max is a quantitative and sensitive index of inotropic changes when these are brought about by stimulating the sympathetic nerves to the heart or the infusion of catecholamines (Furnival, Linden & Snow, 1968*a*, 1971). The ability of  $dP/dt$  max to indicate inotropic changes when such changes are brought about by stimulating the vagus was tested in the duck and toad, animals in which the results of previous investigations (Folkow & Yonce, 1967; Azuma, Hayashi & Matsuda, 1962) have suggested that the vagus innervates the ventricles. A preliminary report of part of this investigation on the dog heart has been given (Furnival, Linden & Snow, 1968*b*).

#### METHODS

Experiments were performed on the dog, duck and marine toad (*Bufo marinus*).

Dogs weighing between 32.7 and 14.1 kg were given a s.c. injection of morphine sulphate (dose 8 mg); one hour later under local anaesthesia a catheter was inserted through a saphenous vein into the inferior vena cava. Each animal was anaesthetized by an i.v. infusion of  $\alpha$ -chloralose 0.1 g/kg body weight (B.D.H. Chemicals, Poole, Dorset); 1 g dissolved in 100 ml. of a solution of sodium chloride (0.9 g/100 ml.). Subsequently a steady state of light anaesthesia was maintained by infusion every 15–30 min of  $\alpha$ -chloralose (1 g/100 ml. in a dose of about 1 ml./kg). The trachea was cannulated and artificial respiration was started using a mixture of 40% oxygen in nitrogen, humidified at room temperature and supplied using an anaesthetic machine incorporating a 'Starling Ideal' pump (Ledsome, Linden & Norman, 1967). When the chest was opened a resistance to expiration was produced by placing the expiratory outlet of the pump under 3 cm H<sub>2</sub>O. The chest was opened in the mid line and the left and right ansae subclaviae were dissected free and crushed with a clamp for 5 min at their origins from the stellate ganglia. An adjustable screw clamp was placed around the descending thoracic aorta. The pericardium was opened widely and bipolar stimulating electrodes applied to the right atrial appendage. The vagal nerves were dissected free in the neck, sectioned and the peripheral ends placed on bipolar stimulating electrodes. The vagal nerves and right atrial appendage were stimulated with monopolar supramaximal impulses using a model S4 stimulator (Grass Instrument Co., Quincy, Mass., U.S.A.).

Pressures in the cardiovascular system were recorded through metal cannulae (Inconel, 1.5 mm bore; Johnson Matthey & Co. London) treated with a solution of dialkyl ammonium chloride (Arquad; Armour Hess Chemicals, Ltd, Leeds) as a non-wetting agent. Aortic blood pressure was recorded from the arch of the aorta through a cannula which was inserted through the right common carotid artery; pressure in the left ventricle was recorded through a cannula which was inserted directly through the apex of the ventricle. Pressure in the trachea was recorded using a manometer connected to the endotracheal airway. Methods of pressure recording and differentiation of the left ventricular pressure wave form (using an analogue differentiator) have been described previously (Furnival, Linden & Snow, 1970).

The signal from the aortic pressure pulse was used to drive a digital cardiometer (Gilford Inst. Co. Inc. Oberlin, Ohio). Oesophageal temperature was recorded

using a thermistor probe (Yellow Springs Inst. Co. Inc., Yellow Springs, Ohio) and was maintained at  $37 \pm 0.5^\circ \text{C}$  by heating lamps above and beneath the animal. The e.c.g. was recorded from electrodes applied to the right fore and left hind limbs.

Samples of femoral arterial blood were withdrawn anaerobically at intervals throughout each experiment and the pH,  $P_{\text{O}_2}$  and  $P_{\text{CO}_2}$  were measured (Norman, Ledsome & Linden, 1965). End-tidal  $P_{\text{CO}_2}$  was monitored continuously by aspirating air from the trachea into an infra-red carbon dioxide analyser (URAS 4, Hartmann and Braun, Frankfurt, Main, West Germany). The arterial  $P_{\text{CO}_2}$  was kept as near as possible to 40 mm Hg by adjusting the respiratory pump and the pH of the arterial blood was maintained at about 7.4 by periodic intravenous infusions of a solution of 0.5 M sodium bicarbonate solution.

In each experiment control records were taken whilst the heart was paced by electrical stimulation of the right atrium at a rate 1 or 2 beats/min above the free heart rate. The vagi were then stimulated at a rate just below that which caused an alteration in heart rate in spite of continual pacing. During vagal stimulation, pressure in the aorta was maintained at the same level as during the initial control period by constricting the aorta with the screw clamp and records of the effects of vagal stimulation were obtained. The pacing of the right atrium was then discontinued and the effects of vagal stimulation on heart rate alone recorded. The vagus was stimulated for a period of 2–3 min. Vagal stimulation was then discontinued, the heart paced again at the same rate as during the initial control period and the period of vagal stimulation and a final set of control records obtained.

The experimental procedures used in the duck and toad were similar to those used in the dog with the following exceptions.

In the ducks (weighing between 3.6 and 2.7 kg) no morphia was used; otherwise the anaesthetic procedure was the same as that used for the dogs. Pressure in the aorta was recorded through a nylon cannula (Portex Surgical quality no. 2) about 10 cm long inserted into the aorta through a wing artery. During stimulation of the vagi, aortic pressure was maintained by the intra arterial infusion of Dextraven 150 (Fisons Pharmaceuticals Ltd, Loughborough), the amount infused being between 5 and 10 ml./kg.

Large marine toads (*B. marinus*) weighing between 0.36 and 0.22 kg were anaesthetized by a subcutaneous injection of a solution of urethane 330 mg/kg body weight. The anaesthetic solution consisted of 2 g urethane, 0.06 g NaCl, 0.007 g KCl, 0.01 g  $\text{NaHCO}_3$ , 0.01 g  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  and 0.1 g glucose dissolved in 100 ml. distilled water. The sternum was removed and the left and right brachial arteries cannulated using nylon cannulae (Portex Surgical quality No. 1). Arterial pressure was recorded through the cannula inserted into the left brachial artery and pressure in the left ventricle through a cannula inserted through the apex in the same manner as used in the dog and duck. During stimulation of the vagi pressure in the aorta was maintained by the intra arterial infusion of Dextraven 150 through a cannula inserted into the right brachial artery. The pH,  $P_{\text{CO}_2}$  and  $P_{\text{O}_2}$  of arterial blood were not measured in the toad. No attempt was made to control body temperature; the ambient temperature was between 21 and 24°C.

## RESULTS

### *Effects of stimulating the vagal nerves in the dog*

In five dogs when recording began about 2 hr after the initial dose of anaesthetic the heart rate was 142 beats/min (mean; range 135–148) the mean aortic pressure was 143 mmHg (mean; range 130–145) and the

maximum rate of rise of pressure in the left ventricles ( $dP/dt$  max) was 3040 mm Hg/sec (mean; range 2200–4200). The pH,  $P_{CO_2}$  and  $P_{O_2}$  of the arterial blood were respectively 7.342 (mean; range 7.32–7.38), 38.5 mm Hg (mean; range 35–41) and 197 mm Hg (mean; range 185–210).

Records obtained in one dog demonstrating the effects of stimulating the left vagus at a rate of 3 stimuli/sec are shown in Fig. 1. During stimulation of the vagus the heart rate was kept constant at 152 beats/min by electrical pacing of the right atrium (free heart rate 152 beats/min) and the mean aortic pressure maintained by partially constricting the aorta with the screw clamp. The records show no significant change in the maximum rate of rise of pressure in the left ventricle. During stimulation of the vagus when the heart was not paced the heart rate decreased to 118 beats/min.

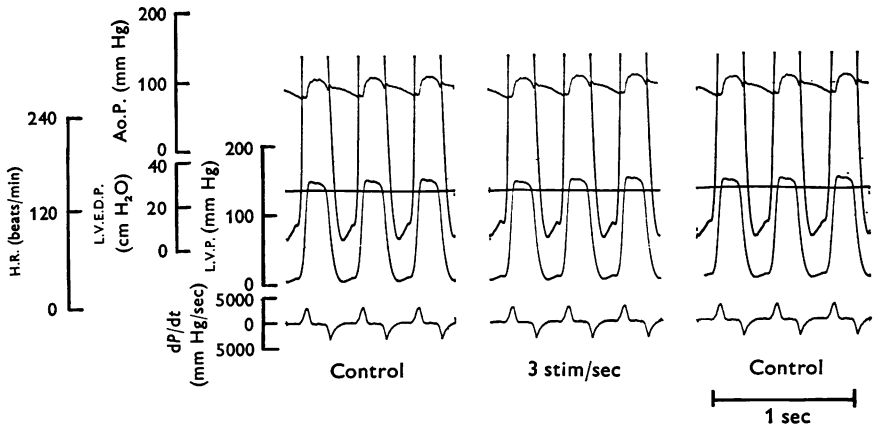


Fig. 1. Records obtained in one dog illustrating the effects of stimulating the left vagus on  $dP/dt$  max. From above downwards pressure in the aorta (Ao.P.), heart rate (H.R.), end diastolic pressure in the left ventricle (L.V.E.D.P.), pressure in the left ventricle (L.V.P.), and the rate of change of pressure in the left ventricle  $dP/dt$ . First panel, control; second panel, during stimulation of the left vagus at 3 stimuli/sec; third panel, post-stimulus control. The heart rate was kept constant throughout at 152 beats/min by pacing the right atrium.

The results obtained in all five dogs are summarized in Table 1. In each dog the maximum frequency of stimulation of the vagus was that which just allowed the contraction of the ventricles to follow the pacing of the atrium. When the heart was not paced, stimulation of the vagus always resulted in a decrease in heart rate, the mean decrease was 57.3 beats/min. The changes in  $dP/dt$  max were small and variable and the over-all range was from +193 mm Hg/sec to -635 mm Hg/sec. However, the small mean change of -189.3 mm Hg/sec was significant ( $P < 0.005$  paired  $t$

test). There were no significant changes in the end diastolic pressure in the left ventricle (mean 9.2 cm H<sub>2</sub>O: s.e. of mean  $\pm$  0.8). The mean control arterial pressure was 142.0 mm Hg (mean; s.e. of mean  $\pm$  4.9) and during stimulation of the vagus was maintained at 138.6 mm Hg (mean; s.e. of mean  $\pm$  4.0). This small decrease in mean arterial pressure is significant ( $0.02 > P > 0.01$ ; *t* test, paired observations) but is not sufficient to

TABLE 1. The effect of stimulating the vagal nerves in the dog on heart rate and  $dP/dt$  max in the left ventricle. Stimulus parameters; frequency of stimulation in impulses/sec (*f*), intensity in volts (*V*), impulse duration in msec (*d*). Right vagus (R), left vagus (L)

Dog no.		Stimulus parameters			Control heart rate (beats/min)	Decrease in heart rate (beats/min)	Control $dP/dt$ max	Change in $dP/dt$ max
		<i>f</i>	<i>V</i>	<i>d</i>				
63	R	5	12	2	139	29	4002	0
	R	5	12	2	139	33	3947	-221
	L	8	12	2	138	28	5106	-469
	L	8	12	2	138	28	4361	-635
65	L	3	12	2	152	34	2898	0
	L	6	12	2	161	31	3174	-55
	R	5	12	2	161	89	2343	+193
	R	5	12	2	160	84	3174	-55
7	L	8	12	2	135	77	2070	0
	L	7	12	2	135	61	1932	-138*
20	L	5	12	2	154	34	2484	-276
	L	9	12	2	146	30	2346	0
	R	12	12	2	146	42	2208	-55
	R	12	12	2	154	68	2346	+83
29	L	3	12	2	164	91	2152	-220
	L	10	12	2	159	74	4692	-607
	L	0.5	12	2	174	76	3762	-552
	L	1	12	2	134	60	2622	0
	L	6	12	2	134	60	2703	-221
	L	7	12	2	164	59	3450	-331
	L	4	12	2	214	106	3864	-414
		Mean			152.4	57.3	3147.7	-189.3
		s.e. of mean			$\pm$ 4.1	$\pm$ 5.3	$\pm$ 170.0	$\pm$ 51.5

$P < 0.005$

\* After i.v. injection of propranolol; dose 0.5 mg/kg.

account for a decrease in  $dP/dt$  max of 189 mm Hg/sec; previous studies (Furnival *et al.* 1970) have shown that a decrease in mean arterial pressure of 3-4 mm Hg results in a decrease in  $dP/dt$  max of only 40 mm Hg/sec. Thus it is concluded that the small observed decrease in  $dP/dt$  max was

a direct effect upon the left ventricle of an increase in activity in the vagal nerves.

The changes in  $dP/dt$  max did not correlate with the changes in heart rate. There was, however, a weak but significant positive correlation ( $r = 0.67$ ;  $P < 0.05$ ) between the control value of  $dP/dt$  max and the magnitude of the decrease in  $dP/dt$  max. It must be emphasised that though in any one dog the fall in  $dP/dt$  max may be increased when the control value is increased, the results from all dogs (Table 1) show that the decrease in  $dP/dt$  max is only weakly correlated with the control value, e.g. in one dog (no. 29) an above average decrease in  $dP/dt$  max of 220 mm Hg/sec was observed when the control value was low, 2152 mm Hg/sec, and in another dog (no. 63) no change in  $dP/dt$  max occurred when the control value was high, 4002 mm Hg/sec.

It has been suggested that the negative inotropic effects brought about by stimulating the cervical vagi are antagonized by a positive inotropic effect which results from stimulation of efferent cardiac sympathetic fibres in the vagal nerve trunk (e.g. Levy, Ng, Martin & Zieske, 1966; Priola & Fulton, 1969; Randall, Pace, Wechsler & Kim 1969). It might be expected therefore, that blockade of the sympathetic nerves to the heart would result in the enhancement of any negative inotropic effect brought about by stimulation of the vagus. In one dog (no. 7) stimulation of the left vagus after sympathetic  $\beta$ -receptor blockade by propranolol (dose; 0.5 mg/kg i.v.) resulted in a decrease in  $dP/dt$  max of only 138 mm Hg/sec, a value which is smaller than the mean value obtained in dogs in which any positive inotropic effects of sympathetic nerve stimulation might have occurred.

Thus in the dog stimulation of the vagus has only a small negative inotropic effect upon the left ventricle. However, a possible explanation of these results obtained in the dog is that  $dP/dt$  max is not a sensitive index of any inotropic changes in the muscle of the left ventricle when brought about by stimulation of the vagus.

#### *Effects of stimulating the vagi in the duck*

Folkow & Yonce (1967) have stated that stimulation of the vagus nerve in the duck has a powerful negative inotropic effect on the heart ventricles. Therefore, it was decided to test the sensitivity of  $dP/dt$  max as an index of the inotropic effect of vagal stimulation on the left ventricle of the duck.

In six ducks when recording began about 2 hr after the initial dose of anaesthetic the heart rate was 321 beats/min (mean; range 155–385) the mean aortic pressure was 106 mm Hg (mean; range 63–170) and the maximum rate of rise of pressure in the left ventricle was 4293 mm Hg/sec (mean; range 2800–8400). In the arterial blood the pH,  $P_{CO_2}$  and  $P_{O_2}$  were

respectively 7.477 (mean; range 7.42–7.56), 29.5 mm Hg (mean; range 25–34) and 269 mm Hg (mean; range 98–358).

Records obtained in one duck illustrating the effects of stimulating the left vagus at 8 stimuli/sec are shown in Fig. 2. The heart rate was kept constant at 162 beats/min by pacing of the right atrium and mean aortic pressure was maintained during the period of vagal stimulation by the infusion of 30 ml. of Dextraven into a wing artery. It may be seen from the records that when heart rate and mean aortic pressure are held constant there is no significant change in  $dP/dt$  max in the left ventricle.

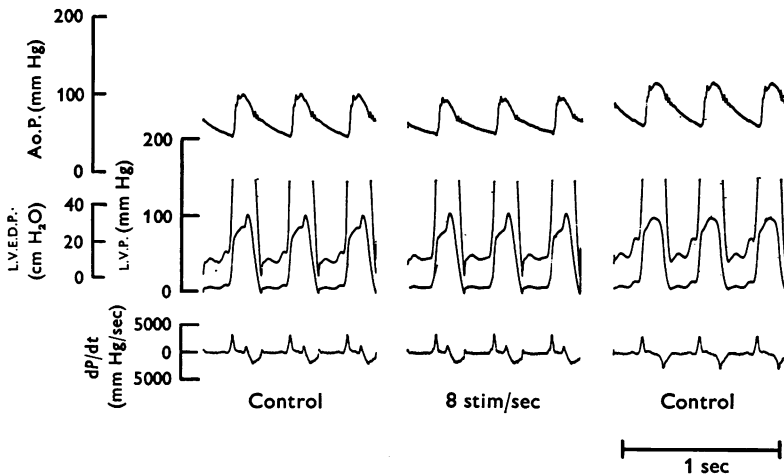


Fig. 2. Records obtained in one duck illustrating the effects of stimulating the left vagus on  $dP/dt$  max. From above downwards pressure in the aorta (Ao.P.), end-diastolic pressure in the left ventricle (L.V.E.D.P.), pressure in the left ventricle (L.V.P.) and the rate of change of pressure in the left ventricle ( $dP/dt$ ). First panel, control; second panel during stimulation of the left vagus at 8 stimuli/sec; third panel, post-stimulus control. The heart rate was kept constant at 162 beats/min throughout by pacing the right atrium.

During stimulation of the vagus when the heart was not paced the heart rate decreased by 73 beats/min to 89 beats/min. Similar results were obtained in five other ducks and the results are shown in Table 2. When the heart was not paced stimulation of either vagus always resulted in a decrease in heart rate; when the heart rate and mean aortic pressure were maintained constant the changes in  $dP/dt$  max were small and variable, the mean change was a decrease of 136 mm Hg/sec (s.e. of mean  $\pm 79.4$ ). This decrease is significant ( $P < 0.05$ ; paired  $t$  test) and is similar to that observed in the series of experiments in the dog. There were no significant changes in mean aortic pressure; there was, however,

a small but significant decrease of 1.9 cm H<sub>2</sub>O (mean; s.e. of mean  $\pm$  0.9) in the end-diastolic pressure in the left ventricle during vagal stimulation. It is concluded from these results in the duck that the vagi have only a relatively small negative inotropic effect upon the ventricles.

TABLE 2. The effect of stimulating the vagal nerves in the duck on heart rate and  $dP/dt$  max in the left ventricle. Stimulus parameters; frequency of stimulation in impulses/sec (f), intensity in volts (V), impulse duration in msec (d). Right vagus (R), left vagus (L)

Duck no.		Stimulus parameters			Control heart rate (beats/min)	Decrease in heart rate (beats/min)	Control $dP/dt$ max (mm Hg/sec)	Change in $dP/dt$ max (mm Hg/sec)
		f	V	d				
2	L	10	15	2	348	84	3220	-140
10	L	8	8	2	162	73	2940	+140
	L	8	8	2	192	91	3164	+56
	R	9	12	2	258	99	8400	+140
	R	10	12	2	258	120	4620	-560
12	L	10	15	2	388	124	3220	+140
18	L	9	15	2	360	204	3920	-280
	L	9	15	2	360	192	4620	-280
20	L	7	12	2	320	116	7920	-140
21	R	10	15	2	324	198	4340	-420
Mean					297.0	130.1	4636.4	-136.4
S.E. of mean					$\pm$ 24.1	$\pm$ 15.7	$\pm$ 620.1	$\pm$ 79.4
								$P < 0.05$

However, in three other ducks in the post stimulus control period, there were large increases in the end-diastolic pressure in the left ventricle which were associated with significant decreases in mean aortic pressure and  $dP/dt$  max. In the post stimulus control period removal of a volume of blood equal to that infused during stimulation of the vagus resulted in the return of the end-diastolic pressure, mean aortic pressure and  $dP/dt$  max to the values obtained in the initial control period. After the above type of response had been obtained once, further stimulation of the vagus resulted in a response which was different from that obtained before the ventricle had been subjected to a high end-diastolic pressure. Thus, in the above three ducks  $dP/dt$  max now decreased during stimulation of the vagus from a control value of 7046 mm Hg/sec (mean; s.e. of mean  $\pm$  1547) to 4834 mm Hg (mean; s.e. of mean  $\pm$  836) and decreased even further in the post stimulus control period to 3545 mm Hg/sec (mean; s.e. of mean  $\pm$  1103). Also during stimulation of the vagus the end-diastolic pressure in the left ventricle increased from a control value of 11.5 cm H<sub>2</sub>O (mean; s.e. of mean  $\pm$  2.3) to 18.7 cm H<sub>2</sub>O (mean; s.e. of



mean  $\pm 1.8$ ) and showed a further increase to 33.3 cm H<sub>2</sub>O (mean; s.e. of mean  $\pm 3.3$ ) in the post stimulus control period. Associated with these changes in  $dP/dt$  max and end-diastolic pressure was a decrease in mean aortic pressure from a control value of 137 mm Hg (mean; s.e. of mean  $\pm 18.6$ ) to 122.7 mm Hg (mean; s.e. of mean  $\pm 18.6$ ) in the post stimulus control period. In the post stimulus control period removal of a volume of blood, about equal to that infused during stimulation of the vagus, until the end-diastolic pressure in the left ventricle was equal to that obtained during the initial control period, did not now result in an increase of the values of  $dP/dt$  max and aortic pressure to their initial control values.

Therefore, it may be concluded that the repeated subjection of the left ventricle to high end-diastolic pressures eventually resulted in failure of the heart muscle and that under these conditions stimulation of the vagus resulted in a negative inotropic response which was greater than that obtained before heart failure occurred.

Thus in the duck, when there is no evidence of heart failure, stimulation of the vagus has only a small negative inotropic effect upon the left ventricle.

The purpose of the above experiments in the duck was to establish the sensitivity of  $dP/dt$  max as an index of inotropic changes in the left ventricle when brought about by stimulation of the vagus. The failure to observe any large change in  $dP/dt$  max in the normal heart made necessary a further test of the sensitivity of  $dP/dt$  max.

#### *Effects of stimulating the vagus nerve in the toad*

The ventricle of the toad (*B. marinus*) is known to be innervated by the vagus and the force of contraction of the ventricle is known to be reduced by either stimulation of the vagus or the injection of acetylcholine (Azuma *et al.* 1962). Therefore, the effects of stimulating the vagus on the ventricle of the toad were studied using a preparation similar to that used in the duck.

In six toads when recording began about 1 hr after the initial dose of anaesthetic the heart rate was 39 beats/min (mean; range 28–43), the mean aortic pressure was 38.7 cm H<sub>2</sub>O (mean; range 29–51) and the maximum rate of rise of pressure in the ventricle was 187 cm H<sub>2</sub>O/sec (mean; range 100–315).

An example of records obtained in one toad illustrating the effects of stimulating the left vagus at 3 stimuli/sec is shown in Fig. 3. During stimulation of the vagus the heart rate was maintained constant, by pacing the sinus venosus, at 36 beats/min and the mean aortic pressure maintained within 4 cm H<sub>2</sub>O of the mean control value by the intra-arterial

infusion of a solution of Dextraven. It may be seen that stimulation of the vagus resulted in a significant reduction in  $dP/dt$  max of 109 cm H<sub>2</sub>O/sec. When the heart was not paced the associated decrease in heart rate was from 30 to less than 1 beat/min. Results obtained in all six toads are shown in Table 3. There was no significant difference between the end-diastolic pressure in the ventricle during the control period and that during stimulation of the vagus. There was a small but significant increase in mean aortic pressure. When not paced the decrease in heart rate was 17.9 beats/min (mean; s.e. of mean  $\pm$  3.3) and when the heart was paced the decrease in  $dP/dt$  max was 57 cm H<sub>2</sub>O/sec (mean; s.e. of mean  $\pm$  9.5).

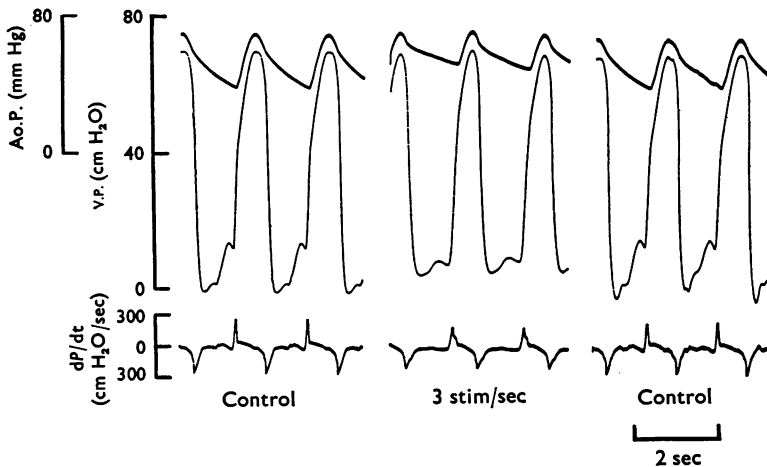


Fig. 3. Records obtained in one toad illustrating the effects of stimulating the left vagus on  $dP/dt$  max. From above downwards, pressure in the aorta (Ao.P.), pressure in the ventricle (v.P.) and the rate of change of pressure in the ventricle ( $dP/dt$ ). First panel, control; second panel, during stimulation of the left vagus at 3 stimuli/sec; third panel, post-stimulus control. The heart rate was kept constant at 36 beats/min throughout by pacing the sinus venosus.

No evidence of heart failure, similar to that observed in the duck, was observed in these experiments. Heart failure in the toad did not occur despite the large effect which stimulation of the vagus had upon the atrial contribution to filling of the ventricle (e.g. Fig. 3) and the fact that intra arterial infusions of dextraven solution had to be given to maintain mean aortic pressure, both conditions which were also present in the duck.

It is concluded that the mean decrease in  $dP/dt$  max of 30% is evidence that  $dP/dt$  max is a sensitive index of inotropic changes in ventricular muscle when these changes are brought about by stimulation of the vagus.

In summary, stimulation of the vagus in the dog sufficient to cause a 38% reduction in heart rate resulted in a reduction in  $dP/dt$  max in the left ventricle of only 6%. Similar results were obtained in the duck in which the reduction in heart rate was 44% and in  $dP/dt$  max 3%. In the toad the reduction in heart rate was 49%, an amount similar to that observed in the dog and duck, whereas the reduction in  $dP/dt$  max of 30% was much greater.

TABLE 3. The effect of stimulating the vagal nerves in the toad on heart rate and  $dP/dt$  max in the ventricle. Stimulus parameters; frequency of stimulation in impulses/sec (f), intensity in volts (V), impulse duration in msec (d). Right vagus (R), left vagus (L)

Toad no.		Stimulus parameter			Control heart rate (beats/min)	Decrease in heart rate (beats/min)	Control $dP/dt$ max (cm H <sub>2</sub> O)	Decrease in $dP/dt$ max (cm H <sub>2</sub> O)
		f	V	d				
2	R	3	5	2	43	43	93	20
	R	3	5	2	36	19	125	10
3	R	1.5	10	2	41	11	112	29
	R	1.5	10	2	39	11	161	61
4	L	3	10	2	30	30	298	109
	L	3	10	2	30	30	277	75
	L	3	10	2	30	15	241	75
5	R	3	10	2	43	16	298	53
	R	5	10	2	44	26	263	130
6	L	2	10	2	34	4	106	28
	L	2	10	2	35	8	153	60
7	R	2	10	2	38	20	176	46
	R	2	10	2	36	0	166	47
Mean					36.8	17.9	189.9	57.0
S.E. of mean					± 1.4	± 3.3	± 20.9	± 9.5

$P < 0.001$

#### DISCUSSION

Whether or not the vagal nerves have a direct negative inotropic effect on the ventricles, apart from any secondary effects resulting from changes in heart rate and aortic pressure, has been controversial since the early experiments of MacWilliam (1888) who claimed to have demonstrated an effect and Bayliss & Starling (1892) who claimed that the vagus had no inotropic effect upon the ventricles of the dog heart. MacWilliam (1930) briefly reviewed previous investigations in which claims were made both for and against a negative inotropic effect and concluded that 'the weakening effects of the vagus, shown by tracings of auricle and ventricle are not explicable by physical changes accompanying the slowing of rhythm, but

connote a definite reduction in the contraction force of the auricular and ventricular muscle'. More recently, Wang, Blumenthal & Wang (1960), claimed to have demonstrated a negative inotropic effect in the dog and Peterson (1950) an effect in man, whereas several other investigators have denied the existence of a negative inotropic effect (e.g. Rushmer 1958; Schreiner, Berglund, Borst & Monroe, 1957; Carlsten, Folkow & Hamberger 1957; Sarnoff *et al.* 1960). Probable reasons for the above contradictory results are that in many experiments factors, such as heart rate and mean aortic pressure known to have an inotropic effect, were not controlled. In some cases the index used to assess the inotropic changes, e.g. systolic pressure in the ventricle or arterial pressure, could have been influenced by changes in the end-diastolic volume of the heart, which resulted in changes in cardiac output and systemic arterial pressure consequent on the mechanism described as Starling's law of the heart. In an attempt to clarify the situation, De Geest *et al.* (1965) investigated the effect of stimulating the vagus on the left ventricle of the dog heart using preparations in which factors known to have an inotropic effect were carefully controlled.

In these experiments the negative inotropic effect was demonstrated in two ways, first as a decrease in the peak pressure developed by an isovolumic left ventricle and secondly as a decrease in stroke work at a given end-diastolic pressure. Examination of the results obtained by De Geest *et al.* (1965) reveals three facts which throw doubt on the conclusion reached, that the vagus has a significant inotropic effect. First, in order to demonstrate a negative inotropic effect the vagus had to be stimulated at frequencies up to 30 stimuli/sec, a rate of stimulation which is usually regarded as maximal and blocks conduction between the atrium and ventricle. Secondly, in their isovolumic preparation, in the only recorded data shown, the control systolic pressure in the left ventricle is only 100 mm Hg and the heart rate is 200 beats/min. In the dog such a high heart rate associated with a low systolic pressure in the ventricle is not typical of a heart in 'good condition'. Thirdly, in a typical experiment quoted in which stroke work was measured and end-diastolic pressure in the left ventricle altered by varying the venous return to the heart, the maximum stroke volume obtained was only 3.5 ml. when the heart rate was 205 beats/min, and the end-diastolic pressure was as high as 20 cm H<sub>2</sub>O. The associated stroke work was only 6 g. *m*, a value which is about one fifth of the expected value in this type of preparation (e.g. Sarnoff *et al.* 1960). Thus it must be concluded that De Geest *et al.* (1965) were only demonstrating the effect of intense vagal stimulation on hearts which were working below the accepted normal range for stroke work at a particular end-diastolic pressure, i.e. the hearts were in failure. Within

such unusual conditions it would seem unreasonable to draw any conclusions about the quantitative significance of the effects of vagal stimulation on the normal heart.

Other investigations (Priola & Fulton, 1969; Daggett, Nugent, Carr, Powers & Harada, 1967) in which vagal stimulation was claimed to have a significant negative inotropic effect, only reported a decrease in  $dP/dt$  max of the order of 10 %, whereas similar changes reported by Harman & Reeves (1968) were claimed to be insignificant. Harman & Reeves (1968) compared the decrease in  $dP/dt$  max in the left ventricle with the decrease in heart rate and the decrease in contractile force of the left atrium. They observed a 70 % decrease in heart rate and contractile force of the right atrium but only a 7.5 % decrease in  $dP/dt$  max in the left ventricle. A characteristic of all the above experiments was that the vagus had to be stimulated at rates of up to 30 stimuli/sec before significant negative inotropic effects could be demonstrated.

A more physiological stimulus was used by Levy *et al.* (1966) who reduced the pressure in the carotid sinus in a preparation in which the activity in the sympathetic nerves had been blocked by an i.v. injection of bretylium tosylate. They observed only a 6.8 % decrease in peak pressure in an isovolumic left ventricle.

Thus in all the above experiments in which the heart was beating within the normal ranges for heart rate, stroke work and end-diastolic pressure or  $dP/dt$  max, the maximum decrease in any of the indices of inotropic change used was only 13 % and on most occasions was of the order 5–10 %. Consistent with this conclusion are the results of Wildenthal, Mierzwiak, Wyatt & Mitchell (1969) who showed that the relationship between the end-diastolic volume of the heart and the stroke volume (measured at constant heart rate and mean aortic pressure) was only slightly altered in the direction indicating a negative inotropic effect when the vagi were stimulated at between 15 and 20 stimuli/sec.

Previous investigations have shown that  $dP/dt$  max in the left ventricle is a sensitive and quantitative index of inotropic changes in the muscle of the left ventricle when these changes are brought about by the infusion of catecholamines or the stimulation of sympathetic nerves (Furnival *et al.* 1968*a*, 1970, 1971). The adequacy of  $dP/dt$  max as an index of inotropic changes in the dog when these changes are brought about by stimulation of the vagal nerves has not previously been established. In this investigation and others in the dog (e.g. Daggett *et al.* 1967; Harman & Reeves, 1968) only small changes of about 5 % were observed in response to stimulation of the vagi. Therefore, it was necessary to investigate the sensitivity of  $dP/dt$  max as an index of changes in the inotropic state in an animal in which it had been demonstrated, by other methods, that the

vagus had a significantly large negative inotropic effect upon the left ventricle. The results obtained by Folkow & Yonce (1967) in the duck suggested that the duck might be a suitable animal in which to test  $dP/dt$  max. However, the results obtained here in the duck were similar to those obtained in the dog. Therefore, further experiments were carried out in the large toad (*Bufo marinus*), since it is known that the vagus innervates the ventricle in this animal. The results obtained demonstrate that  $dP/dt$  max in the ventricle is a sensitive and quantitative index of negative inotropic changes in the heart muscle when these changes are brought about by stimulation of the vagus.

In the investigation reported here the inotropic effects of changes in heart rate and mean aortic pressure (Furnival *et al.* 1970) were prevented from occurring during the stimulation of the vagus by maintaining heart rate and mean aortic pressure at the same levels as those observed during the control periods. Thus only the direct effects on the ventricle of stimulating the vagus were observed, which in the event were very small in the dog and duck, a decrease in  $dP/dt$  max of the order of 5–6 %, but larger in the toad, of the order of 30 %. When it is remembered that stimulation of the sympathetic nerves or the infusion of catecholamines can produce changes in  $dP/dt$  max of the order of 300 % (e.g. Furnival *et al.* 1968*a*, 1970, 1971) it must be concluded that the negative inotropic effect on the dog and duck heart, brought about by stimulation of the vagus, is not physiologically significant.

However, the results obtained in the duck are of importance since they illustrate that the normally small negative inotropic effect on the ventricle of stimulating the vagus may be enhanced if the ventricle has been subjected previously to a high end-diastolic pressure and shows evidence of failure. Evidence of failure was taken as being that further increases in end-diastolic pressure produce a reduction in  $dP/dt$  max and aortic blood pressure or that reduction in end-diastolic pressure by bleeding produces increases in these parameters. The results obtained in the duck are contrary to those obtained by Folkow & Yonce (1967) who concluded from their observations that the vagus had a powerful negative inotropic effect on the ventricles. They also observed that during stimulation of the vagus, when the heart rate was held constant, the end-diastolic pressure in the ventricle increased especially when the mean aortic pressure was also maintained constant. In this present investigation there was a small mean decrease in the end-diastolic pressure in the ventricle and only in those ducks which showed evidence of heart failure did the end-diastolic pressure increase during stimulation of the vagus. In the three ducks showing evidence of heart failure, further tests of the effects of stimulation of the vagus now resulted in significant negative inotropic changes.

Thus a possible explanation of the results obtained by Folkow & Yonce (1967) could be that the hearts studied had been or were in failure as a result of the repeated subsection of the left ventricle to high end-diastolic pressures, brought about by the infusions of the fluid required to maintain aortic pressure during vagal stimulation.

In conclusion the results obtained in the toad demonstrate that  $dP/dt$  max is an adequate index of negative inotropic changes in the left ventricle when these are brought about by stimulation of the vagus. In the dog and duck, the observed negative inotropic effects were small and variable and in the normal heart cannot be regarded as significant when compared with the effects observed in the toad. However, the results obtained in the duck and those of previous investigations in the dog (e.g. De Geest *et al.* 1965) demonstrate that in the failing heart the negative inotropic effect of stimulating the vagus becomes relatively more important.

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