Table 1. Effects of adenine nucleotides on procaine action in normal calcium-free Ringer.

Nucleotide	Concn. ($\times 10^{-3}M$)	Decrease of action potential amplitude* (%)	
		Normal	EDTA-Ringer
None (control)	0	70	85
ATP	1	3	68
ADP	1	7	—
AMP	1	6	74
ATP	10	0	55
AMP	10	0	58

* Mean value of seven experiments; 30-minute treatment + $5 \times 10^{-3}M$ procaine in each case.

of these same nucleotides on procaine action is severely reduced in the calcium-free Ringer solution. As little as $1 \times 10^{-3}M$ of the nucleotide almost completely antagonizes the action of $5 \times 10^{-3}M$ of procaine in the presence of a normal calcium-ion concentration.

Several organic phosphates were also tested as procaine antagonists but were ineffective in concentrations up to $10 \times 10^{-3}M$. These were CrP, ribose-5′-phosphate, and phenylphosphate. Also ineffective were adenosine and sodium pyrophosphate.

In other experiments we found that nucleotides can reverse a procaine-induced depression of action potential amplitude. For example, nerves were treated for 30 minutes with $5 \times 10^{-3}M$ procaine in normal Ringer, then placed in procaine-Ringer containing $10 \times 10^{-3}M$ ATP. Despite the continuous presence of procaine, the action potential amplitudes returned to those of the control or to within 10 to 20 percent of that at the end of 60 minutes. Even as little as $1 \times 10^{-3}M$ ATP caused an increase in the action potential to within 40 percent of control after 60 minutes. In the absence of ATP, complete recovery from the effects of $5 \times 10^{-3}M$ procaine after 30 minutes of treatment required about 2.5 hours, even though the procaine was absent during the recovery period and the normal Ringer solution was periodically changed.

Of the three adenine nucleotides used, only ATP caused a measurable increase in spike amplitude of nerves bathed in normal Ringer solution. At $10 \times 10^{-3}M$ concentration, the average increase in six nerves was only 9 percent. This effect was probably caused by the addition of $20 \times 10^{-3}M$ sodium to the Ringer solution because ATP was used as the disodium salt. In fact, the spike amplitude was increased to the same extent when $20 \times 10^{-3}M$ Na_2SO_4 was added to the Ringer. Also $5 \times 10^{-3}M$ procaine depresses the spike amplitude to the same degree in a Ringer containing extra sodium ($20 \times 10^{-3}M$ Na_2SO_4 added) as it does in normal Ringer solution, indicating that the effects of ATP or ADP on procaine action are not caused by high extracellular sodium.

There is evidence that the depressant action of procaine on nerve excitability is due to competition with extracellular sodium (*4*). Accordingly, antagonism of procaine action could result from a specific ATP effect on sodium influx. To test this possibility, nerves were immersed for about 5 hours in solutions containing $20 \times 10^{-3}M$ sodium-ion concentration (remainder replaced by choline Cl). Action potential amplitude in each of these nerves was depressed by at least 50 percent. No increase in these potentials was observed after replacing the nerve bath by another solution containing $10 \times 10^{-3}M$ ATP and $20 \times 10^{-3}M$ sodium. Thus, it seems unlikely that the ATP effect on procaine-treated nerve is produced through a direct influence on sodium-ion transport.

The ATP antagonism of procaine action probably does not depend on phosphate splitting and energy transfer because AMP is as effective an antagonist as ATP, and CrP is completely ineffective. The evidence does indicate that the ATP effect is associated with the presence of some free extracellular calcium. Perhaps calcium is required for the binding of ATP to certain membrane sites (*2*), and nerve-impulse initiation and conduction may be associated with displacement of calcium from these sites (*6*), causing the release of membrane ATP (*7*); these events would be followed by protein conformation changes (*8*) and increased sodium permeability. Therefore our results could indicate that procaine depresses nerve excitation by interfering with calcium and ATP mobilization or by causing release of ATP and other nucleotides from the excitable membrane.

ALBERT S. KUPERMAN
MICHIKO OKAMOTO
ANNETTE M. BEYER
WILLIAM A. VOLPERT
Department of Pharmacology, Cornell University Medical College, New York 10021

References and Notes

1. Abbreviations: ATP, adenosine-5′-triphosphate; ADP, adenosine-5′-diphosphate; AMP, adenosine-5′-monophosphate; CrP, creatine phosphate; tris, tris (hydroxymethyl) aminomethane; EDTA, ethylenediaminetetraacetate.
2. M. Okamoto, A. Askari, A. S. Kuperman, *J. Pharmacol.*, in press.
3. J. Aceves and X. Machne, *ibid.* **140**, 38 (1963).
4. G. A. Condouris, *ibid.* **131**, 243 (1961).
5. Sigma Chemical Co.
6. J. M. Tobias, D. P. Agin, R. Pawlowski, *J. Gen. Physiol.* **45**, 989 (1962).
7. L. G. Abood, K. Koketsu, S. Miyamoto, *Am. J. Physiol.* **202**, 469 (1962).
8. M. Luxoro, E. Rojas, E. Wittig, *J. Gen. Physiol.* **46**, 1109 (1963).
9. Supported by USPHS grant B-3597(C).

17 March 1964

Negative Inotropic Effect of the Vagus Nerves upon the Canine Ventricle

Abstract. *In an innervated, paced, left heart preparation in which the left ventricle contracted against a constant volume of incompressible fluid (isovolumetric preparation), electrical stimulation of the distal end of either sectioned cervical vagus nerve decreased the peak tension generated by the left ventricle. The vagi, therefore, exert a negative inotropic effect directly upon the ventricular myocardium.*

There is general agreement that the vagus nerves have a profound depressant influence upon the pacemaker cells, the conduction system, and the atrial myocardium in mammalian hearts. Although there have been reports (*1*) that the vagi also exert a negative inotropic effect upon the ventricular myocardium, the preponderant view (*2, 3*) is that efferent vagal stimulation has no direct influence upon ventricular contractility. Interpretation of many of the earlier studies has been complicated by the concomitant actions of these parasympathetic nerves upon heart rate, coronary perfusion pressure, and the transport function of the atria. In the study reported here, these difficulties were circumvented by using a paced, innervated left ventricle preparation in which the ventricle contracted against a fixed volume of incompressible fluid (isovolumetric preparation). Under these conditions, efferent vagal stimulation consistently elicited an appreciable negative inotropic response.

Seven experiments were performed upon mongrel dogs under morphine-chloralose-urethane anesthesia. After ligation of the descending thoracic

aorta, the cephalic portion of the animal and the coronary vascular bed were perfused at a constant pressure with artificially arterialized blood from a rotating-disk oxygenator through a cannula inserted in the left subclavian artery. Venous blood was returned by gravity to the oxygenator through a large-bore cannula inserted in the right atrium and ventricle. Through a small incision in the apex of the left ventricle, a latex balloon was introduced into the left ventricular cavity. It was filled with sufficient isotonic saline (5 to 15 ml) to result in the production of a peak pressure of 80 to 100 mm-Hg during systole. The presence of this volume of fluid produced a positive diastolic pressure, which remained constant in any given experiment. Drains in the left atrium and left ventricle prevented accumulation of blood in the left ventricular cavity. Phasic pressure in the balloon was registered on a Sanborn recorder, by means of strain gauges.

The upper two tracings in Fig. 1 show the effects of electrical stimulation (*4*) of the distal end of the left cervical vagus on heart rate and left ventricular pressure in a representative experiment before pacing. Stimulation with 3 v or 5 v at frequencies of 3 or 5 cy/sec, and a pulse duration of 5 msec for a period of 30 seconds (top two tracings in Fig. 1, *A*, *B*, *D*, and *E*) produced a sinus bradycardia and a decrease in the peak systolic pressure in the left ventricle. The magnitude of the changes in rate and pressure varied with the strength and frequency of the stimulus. A complete atrioventricular block occurred when the nerve was stimulated with 3 or 5 v at a frequency of 10 cy/sec (top two tracings in Fig. 1, *C* and *F*).

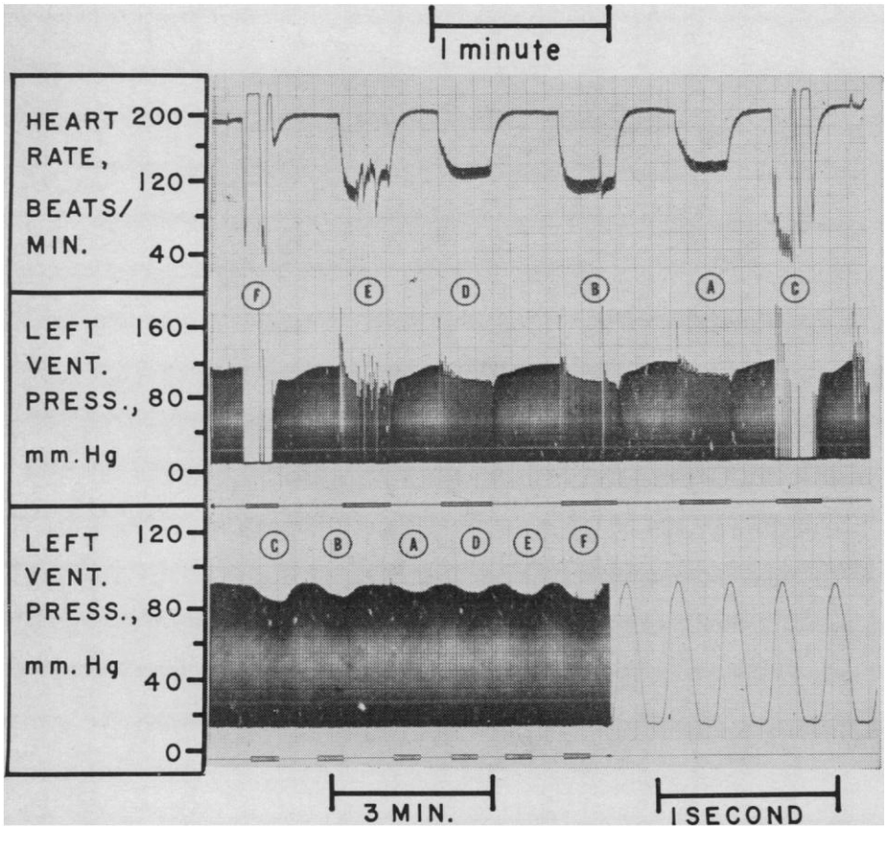

Fig. 1. The upper two tracings show the effects of electrical stimulation of the distal end of the left cervical vagus upon the rate and intraventricular pressure in the unpaced heart. The lowest tracing shows the influence of left vagal stimulation upon intraventricular pressure in the paced heart. Left atrium and right ventricle paced electrically at 206 beats per minute. Coronary perfusion pressure constant at 120 mm Hg. Volume of fluid in latex balloon in left ventricle, 12 ml. Heavy bars below the second and below the lowest tracings indicate the duration of stimulation. *A*, *B*, and *C*: stimulus intensity, 3 v; frequency, 3, 5, and 10 cy/sec, respectively. *D*, *E*, and *F*: stimulus intensity, 5 v; frequency, 3, 5, and 10 cy/sec, respectively. Duration of all pulses, 5 msec, applied for 30 seconds.

Immediately after these tracings had been registered, the heart was paced at a constant rate slightly above the spontaneous rate by simultaneous electrical stimulation of the left atrial appendage and right ventricle. The effects of stimulation of the efferent left vagus (when stimuli of the same intensities, frequencies, and durations were used, but in a different sequence) upon left ventricular pressure are displayed in the bottom tracing of Fig. 1. Those stimuli which previously had caused sinus bradycardia in the unpaced heart caused a definite decrease in the peak systolic pressure of the left ventricle (*A*, *B*, *D*, and *E*). A more marked effect was seen with the stimuli which produced a complete atrioventricular block in the unpaced heart (*C* and *F*). No changes in left ventricular pressure at the end of diastole were observed.

Similar observations were made in all seven consecutive experiments, but the relative changes in the peak systolic pressure of the left ventricle in the paced heart in the experiment depicted in Fig. 1 were the smallest in the entire series. Supramaximal stimulation (5 to 50 v, 50 cy/sec, 5-msec pulses) of the distal end of the cervical vagi in the paced heart produced a mean decrease in left ventricular systolic pressure of 25.4 ± 9.9 (mean ± S. D.) percent, and these changes were statistically significant ($p < .001$). In the paced hearts, no differences were observed between the effects of stimulation of the right and left vagi upon the peak pressure in the left ventricle.

A decrease in the peak systolic pressure of the left ventricle, when the volume of the ventricle at the end of diastole, the heart rate, and the perfusion pressure of the coronary and cephalic vascular beds were kept constant, clearly indicates a decrease of left ventricular contractility during vagal stimulation. The observation that stimuli which cause only a slowing of the sino-atrial pacemaker depress left ventricular systolic pressure in the paced heart suggests strongly that the vagi continuously exert a tonic negative inotropic effect upon the ventricular myocardium. In these experiments, such stimuli elicited rates equivalent to those which existed prior to transection of the vagi, and to those which prevail in unanesthetized animals.

Although our conclusions disagree with the prevalent current concepts (*2*, *3*), they are consonant with (i)

certain anatomical evidence which, although scanty, does indicate that vagal fibers do reach the ventricles (*5*), (ii) the report that the ventricular rate may diminish during vagal stimulation in dogs with complete atrioventricular block (*6*), (iii) the well-known depressing action of acetylcholine upon ventricular contractility (*2*, *6*), and (iv) the observation that acetylcholine is synthesized in the ventricles as well as in the atria (*7*).

HILAIRE DEGEEST
MATTHEW N. LEVY
HARRISON ZIESKE
Research Division, St. Vincent Charity Hospital, Cleveland, Ohio

References and Notes

1. J. A. MacWilliam, *Quart. J. Exptl. Physiol.* **20**, 149 (1930); H. Wang, M. R. Blumenthal, S. C. Wang, *Circulation Res.* **8**, 271 (1960).
2. G. L. Schreiner, E. Berglund, H. G. Borst, R. G. Monroe, *Circulation Res.* **5**, 562 (1957).
3. A. N. Drury, *Heart* **10**, 405 (1923); A. B. Denison and H. D. Green, *Circulation Res.* **6**, 633 (1958); S. J. Sarnoff, S. K. Brockman, J. P. Gilmore, R. J. Linden, J. H. Mitchell, *ibid.* **8**, 1108 (1960); H. Schaefer, *Physiol. Rev.* **40** (Suppl. 4), 213 (1960).
4. Grass stimulator, model S4.
5. K. T. Tcheng, *Am. Heart J.* **41**, 512 (1951); G. A. G. Mitchell, R. Brown, F. B. Cookson, *Nature* **172**, 812 (1953).
6. M. Eliakim, S. Bellet, E. Tawil, O. Muller, *Circulation Res.* **9**, 1372 (1961).
7. E. Bulbring and J. H. Burn, *J. Physiol. (London)* **108**, 508 (1949); M. Day, *ibid.* **134**, 558 (1956).
8. Supported by PHS grant HE-07724-02.

12 March 1964

Monoamine Oxidase Inhibitors: Augmentation of Pressor Effects of Peroral Tyramine

Abstract. *Monoamine oxidase inhibitors markedly enhance the oral pressor potency of tyramine by preventing it from being destroyed by the monoamine oxidase normally present in liver and intestine. Since certain types of cheese contain high concentrations of tyramine, they should not be eaten by patients during treatment with a monoamine oxidase inhibitor.*

A fairly common side effect associated with therapy with monoamine oxidase inhibitors is orthostatic hypotension (*1*, *2*). Recently, a number of investigators have reported the occurrence of paradoxical hypertension after administering a monoamine oxidase inhibitor. This report is concerned with a mechanism that may be in part responsible for this effect.

Adult male albino rats (Wistar strain) weighing 200 to 400 g were treated orally with various doses of tranylcypromine once a day for 2 days, or pargyline or iproniazid, once a day for 3 days. After treatment the rats were anesthetized intravenously with pentobarbital (45 mg/kg) and arterial blood pressure was recorded from the carotid artery. The arterial pressure was permitted to stabilize over a 15-minute interval after which the rats were treated with various doses of tyramine by gastric intubation. Blood pressure was recorded continuously until either a maximum change in pressure had been achieved or until a 20-minute period had elapsed after treatment with tyramine. Mean changes in systolic, mean, and diastolic pressure were determined for each dose of each drug. A control group of rats was tested in the same manner as the treated rats. The results of these experiments are summarized in Table 1.

In control rats it was found necessary to increase the dose of tyramine above 25 mg per kilogram of body weight in order to evoke a pressor response significantly greater than that caused by gastric intubation with an equivalent volume of saline. In general, the increase in blood pressure which occurred after tyramine administration came on approximately 5 to 10 minutes after its gastric intubation.

All of the monoamine oxidase inhibitors tested permitted a reduction in the dose of tyramine necessary to evoke a significant pressor response. Although the time for onset of the pressor response was similar to that for the controls, the duration of the response was generally more prolonged.

The data clearly demonstrate that monoamine oxidase inhibitors can markedly potentiate the pressor response to orally administered tyramine. Presumably this is accomplished by inhibition of liver or intestinal monoamine oxidase, or both. Thus inhibition of monoamine oxidase may permit doses of tyramine which would normally be destroyed in the liver or intestine to enter the systemic circulation and evoke a pressor response through the release of norepinephrine (*3*). In the absence of a monoamine oxidase inhibitor, 4 to 16 times as much tyramine is required to evoke a pressor response. It is assumed that such relatively high doses of tyramine eventually block monoamine oxidase activity by providing excessive substrate which, in turn, permits sufficient tyramine to penetrate the systemic circulation, thereby evoking a pressor response.

Table 1. Effect of monoamine oxidase inhibitors on the pressor response to orally administered tyramine. A minimum of six rats were tested for each dose of tyramine.

Dose of tyramine (mg/kg)	Mean pressor response (mm-Hg) ± S.E.		
	Systolic	Mean	Diastolic
	Normal (no inhibitor)		
0	8.6 ± 4.5	5.1 ± 4.5	5.0 ± 4.1
25.0	5.0 ± 2.6	1.8 ± 2.1	1.5 ± 2.2
50.0	34.7 ± 11.2	23.3 ± 7.0	17.8 ± 5.1
	Tranylcypromine 0.5 mg/kg per day (2 days)		
3.0	5.6 ± 2.6	5.4 ± 1.8	5.0 ± 1.6
6.0	10.7 ± 4.8	9.8 ± 4.2	8.3 ± 4.0
	Tranylcypromine 1.0 mg/kg per day (2 days)		
3.0	9.8 ± 3.2	7.7 ± 2.8	7.6 ± 2.8
6.0	*54.4 ± 10.1	*40.8 ± 6.8	*37.5 ± 5.6
12.5	*46.9 ± 13.4	*36.5 ± 9.7	*31.8 ± 8.5
25.0	*47.5 ± 5.9	*34.2 ± 4.5	*27.5 ± 4.2
	Tranylcypromine 2.0 mg/kg per day (2 days)		
3.0	*26.6 ± 8.0	*28.4 ± 16.4	*26.0 ± 14.3
6.0	*44.2 ± 12.4	*36.7 ± 11.9	*37.5 ± 11.4
	Iproniazid 50 mg/kg per day (3 days)		
3.0	20.0 ± 8.7	16.6 ± 8.0	15.0 ± 7.6
6.0	*30.0 ± 12.4	*22.2 ± 8.7	*19.2 ± 7.4
12.5	*36.0 ± 15.3	*29.0 ± 13.6	*28.0 ± 12.1
	Pargyline 100 mg/kg per day (3 days)		
6.0	8.7 ± 4.2	8.5 ± 3.7	7.5 ± 3.6
12.5	*59.2 ± 13.7	*52.5 ± 9.1	*45.8 ± 9.1

* Significant increase over control response to 25 mg/kg of tyramine.