Table 2. Statistical results between groups (Student's *t*-test).

Group	Group 2	Group 3	Group 4
1	< 0.01	< 0.01	< 0.01
2		$< .05$	$< .05$
3			$> .05$

would alter the analeptic effect of amphetamine.

We measured the sleeping time of 80 female mice of the same age which had been kept in groups of 10 per cage prior to the injection of drugs. The animals, each weighing between 22 and 28 g, were divided at random into four groups as shown in Table 1. We gave each animal three injections intraperitoneally in rapid succession, taking a total time of less than 15 seconds, then placed them in individual containers. Twenty animals per day were used so that all animals were injected within ½ hour and at the same time each day. Care was taken to include animals from each group in each day's experiment. As soon as the animals were completely anesthetized they were placed on their backs. The first two times they righted themselves, they were immediately turned on their backs again. Sleeping time was taken as the time from completing the third injection till the animal had righted itself for the third time.

The results summarized in Table 1 indicate that eserine (salicylate) does have an analeptic effect on mice injected with pentobarbital (compare groups 1 and 3). However, eserine also antagonized the analeptic effect of amphetamine on mice injected with pentobarbital (compare groups 2 and 4). A summary of the statistical significance between groups is shown in Table 2. A separate experiment demonstrated that neostigmine had no effect on pentobarbital sleeping time.

It has been reported that eserine and amphetamine act on different cells to produce their characteristic electroencephalographic desynchronization (*3*), and there is evidence that adrenergic and cholinergic drugs have opposing effects on some cells in the midbrain (*4*). In the light of these findings the results reported here are interpreted as indicating that eserine and amphetamine act on separate neuronal pools, the activation of either one resulting in an early arousal from pentobarbital sleep. Eserine, however, either through those cells activated by it or by direct action, has an inhibitory influence on the amphetamine-activated neuron pool.

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References and Notes

1. R. P. White and L. D. Boyajy, *Proc. Soc. Exptl. Biol. Med.* **102**, 479 (1959).
2. S. Y. Arbusov, *Fiziol. Zh. SSSR* **36**, 704 (1950).
3. V. G. Longo and B. Silvestrini, *J. Pharmacol. Exptl. Therap.* **120**, 160 (1957); R. Y. Il'yuchenok and M. D. Mashkovskii, *Fiziol. Zh. SSSR* **47**, 1352 (1961).
4. P. B. Bradley and J. H. Wolsteneroft, *Nature* **196**, 840 (1962); J. Courville, J. Walsh, J. P. Cordeau, *Science* **138**, 973 (1962).
5. This study was supported by USPHS grant 2M 7082.

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Procaine Action: Antagonism by Adenosine Triphosphate and Other Nucleotides

Abstract. *Appropriate concentrations of adenosine tri-, di-, and monophosphate antagonize the depressant action of procaine on isolated nerve. Some calcium ion is required in the external solution in order for these nucleotides to produce their maximum effects. When applied either to normal or sodium deficient nerves, the nucleotides do not increase action-potential amplitude.*

The actions of ATP (*1*) and other nucleotides on excitable cells have been under investigation in this laboratory. These compounds antagonize the neural effects of calcium deficiency in the presence of about one-hundredth the normal external calcium-ion concentration (*2*). Evidence indicates that some calcium is needed to form complexes with the nucleotides in external solution, and these complexes presumably are bound to certain membrane sites. While our experiments were in progress, it was reported that a fivefold increase in the external calcium-ion concentration opposes the depressant action of procaine on nerve cells (*3*). In view of a possible interaction between calcium and nucleotides in the neuronal membrane, it seemed important to determine whether the procaine effect is also antagonized by nucleotides.

The isolated sciatic nerve of the frog, *Rana pipiens*, was used at room temperature (about 22°C) in Ringer solution (*p*H 7.2) containing $110.88 \times 10^{-3}M$ NaCl, $2.0 \times 10^{-3}M$ KCl, $1.8 \times 10^{-3}M$ $CaCl_2$, $0.1 \times 10^{-3}M$ NaH_2PO_4, and $2.0 \times 10^{-3}M$ $NaHCO_3$. All drugs were dissolved in this Ringer solution and then added to the nerve chamber. The addition of nucleotides to the Ringer caused a decrease in *p*H, and the *p*H was readjusted to 7.2 by adding tris or NaOH; the experimental results were the same regardless of which base was used. When relatively high nucleotide concentrations were prepared ($> 10 \times 10^{-3}M$), tris was always used for *p*H adjustment because high concentrations of sodium ion antagonize procaine action (*4*). The *p*H of the nerve bath was periodically checked during the course of an experiment, and it was never found to vary by more than 0.1. Monophasic action potentials were recorded in air at periodic intervals by conventional techniques. Stimulus voltage and duration was supramaximal for the rapidly conducting A fibers, and the stimulus was applied once every 2 seconds. The entire length of nerve was exposed to test solutions, the segments lying across both the stimulating and recording electrodes. The organic phosphates used were the highest purity obtainable (*5*), and calcium contamination was at most 0.2 percent. Except for AMP, all phosphates were used as sodium salts; AMP was the free acid. The EDTA was the disodium salt in a $5 \times 10^{-3}M$ concentration.

Curves of dose-response and time-action were determined for procaine dissolved in normal and in EDTA-Ringer solutions. For the purpose of testing antagonism of procaine action by nucleotides, we selected a procaine dose which produces close to maximum depression of action potential amplitude, but does not completely abolish it; this dose is $5 \times 10^{-3}M$ (Table 1). After immersing a nerve for 30 minutes in $5 \times 10^{-3}M$ procaine, application of ten times the control stimulus voltage and duration failed to increase the action potential amplitude.

The results of the nucleotide antagonism experiments are summarized in Table 1. In normal Ringer, adenine nucleotides prevent the depressant action of procaine. However, the effect

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.

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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).

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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