nents of seed mixtures on an ear are compared. Total seed weight per ear is usually not influenced by paternal genotype (*4*).

Relative seed weight for each cross was expressed as follows:

Relative seed weight =

$$\frac{\overline{X}_{fi}}{\overline{X}_{si}} + \frac{\overline{X}_{fi+1}}{\overline{X}_{si+1}} \cdots + \frac{\overline{X}_{f5}}{\overline{X}_{s5}}$$

Where $\overline{X}_{fi}$ is the average seed weight with faster pollen as staminate parent in segment i; i = 1, for the apical segment and i = 5 for the basal segment; $\overline{X}_{si}$ is the average weight of seeds with the slower staminate parent. This procedure corrected for any differences resulting from position on the ear. (Otherwise, the value for the faster type would be distorted because basal seeds tend to be heavier than others.)

For each pollen mixture, relative speed of growth of pollen tubes was plotted against relative seed weight (Fig. 1). The greater the relative pollen tube speed, the greater will be the relative weights of the resultant seeds; when there is no differential in pollen tube speed, there is no differential in seed weight.

The unmixed pollinations indicated not only that all pollen types were capable of reaching basal kernels, but also confirmed reports (*4*) that the paternal genotype does not influence total kernel weight per ear. Thus, differences within seed mixtures result from competitive interactions between seeds.

Three possible mechanisms may be considered to explain the correlation between pollen tube speed and weight (or competitive ability) of resultant seeds: (i) faster pollen tubes give resultant zygotes a temporal advantage over zygotes of later fertilizations, (ii) a paternal genotype which produces rapidly growing pollen tubes produces relatively vigorous zygotes, (iii) gametes from pollen tubes that function well in a particular stylar environment give rise to heterotic zygotes. The argument of a temporal advantage is greatly weakened by a study (*5*) in which components of a mixture were applied 1 week apart to a single corn inflorescence. Pollen giving rise to relatively large seeds when in mixtures did so despite the delay in application. The second possibility, that paternal genotype gives an overwhelming advantage to both pollen tubes and resultant zygotes, may be correct, but when the same mixture is applied to different pistillate types, the competitive abilities of pollen types sometimes change drastically. This suggests significant interactions between pollen tube and stylar tissues. Stylar effects upon the outcome of pollen tube competition are well established (*6*). That such interactions should parallel those between paternal and maternal genotypes in the zygote is reasonable since style and egg should share any genetic factors for which the maternal parent is homozygous. The third, and most likely, explanation for the observed results refers to these interactions. Thus, some genetic factors are expressed in both phases of the life cycle. This evidence is interesting in that it suggests that gametophytic competition in some plants may have a significant effect upon sporophytic characteristics. The implications of this evidence include the possibility that the gametophytic phase in angiosperms may serve to eliminate deleterious genetic traits. Also suggested is a possible explanation for the heterozygosity observed in some plants even after many generations of inbreeding.

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Adenosine 3′,5′-Monophosphate in Nervous Tissue: Increase Associated with Synaptic Transmission

Abstract. *Brief periods of stimulation of the preganglionic nerve fibers produced a severalfold increase in the content of adenosine 3′,5′-monophosphate in superior cervical sympathetic ganglia, whereas postganglionic stimulation did not. These and other experiments indicated that the increased concentrations of adenosine 3′5′-monophosphate were closely associated with the process of synaptic transmission. This increase occurred primarily in postsynaptic cells.*

There is considerable evidence which suggests that adenosine 3′,5′-monophosphate (cyclic AMP) may be involved in regulation of metabolism and function in the nervous system. Several reviews of this subject have appeared recently (*1*). Included in the evidence is the finding that electrical stimulation of brain slices results in an increase in the concentration of cyclic AMP (*2*). However, it was not possible to conclude from those experiments whether the effect on

Table 1. Effect of preganglionic stimulation on the content of cyclic AMP in rabbit superior cervical sympathetic ganglia. One ganglion from each rabbit was stimulated at a frequency of 10 per second. The contralateral ganglion served as an unstimulated control. The data have been calculated as the mean ± standard error for the number (N) of rabbits indicated in the second column. In the last column, the concentration of cyclic AMP in the stimulated ganglia is expressed as the percentage of that in the unstimulated control ganglia. Temperature, 33°C.

Duration of stimulation (min)	N	Cyclic AMP (picomoles per milligram of protein)			Percentage of control
		Unstimulated ganglion	Stimulated ganglion	Absolute increase	
0.5	5	23.2 ± 5.5	43.4 ± 11.2	20.3 ± 13.0	249 ± 113
1.0	5	13.1 ± 1.3	61.8 ± 6.1	48.7 ± 5.2	479 ± 39
2.0	11	18.0 ± 1.7	70.2 ± 9.1	52.1 ± 8.2	399 ± 40
4.0	4	14.2 ± 2.9	57.0 ± 3.0	43.5 ± 4.3	483 ± 136
8.0	4	16.4 ± 1.0	72.0 ± 4.5	55.7 ± 4.6	445 ± 41

Table 2. Effect of stimulation on the content of cyclic AMP in various preparations of isolated peripheral nervous tissue. Preparations were stimulated at a frequency of 10 per second. The contralateral tissue served as the unstimulated control in each experiment. The data have been calculated as the mean ± standard error for the number (*N*) of rabbits indicated in the fourth column.

Nervous tissue	Temperature (°C)	Duration of stimulation (min)	*N*	Cyclic AMP (picomoles per milligram of protein)		
				Unstimulated	Stimulated	Absolute increase
Superior cervical ganglion, preganglionic stimulation	26	2	5	17.1 ± 2.5	40.9 ± 3.5	23.8 ± 1.9
Superior cervical ganglion, postganglionic stimulation	26	2	5	14.2 ± 1.4	13.7 ± 1.4	−0.5 ± 0.4
Nodose ganglion	33	2	7	17.5 ± 5.3	21.2 ± 4.6	3.7 ± 5.8
Cervical vagus	33	2	5	23.4 ± 3.0	19.9 ± 3.2	−3.9 ± 5.0
Superior cervical ganglion, preganglionic stimulation	33	1	5	13.1 ± 1.3	61.8 ± 6.1	48.7 ± 5.2
Superior cervical ganglion, preganglionic stimulation, 1 m*M* hexamethonium chloride plus 0.1 m*M* atropine sulfate	33	1	4	17.6 ± 1.4	22.0 ± 1.4	4.4 ± 1.6

the concentration of cyclic AMP reflected what might be seen under more physiological conditions of nervous activity, nor could it be ascertained at what level of cellular organization this biochemical effect had occurred. In the hope of answering these questions, we studied the effect of nervous activity on the concentration of cyclic AMP in various types of peripheral nervous tissue. Such tissues are amenable to experimental manipulation under relatively physiological conditions. We determined the cyclic AMP content of these small tissues by using a sensitive analytical method for cyclic AMP recently developed in our laboratory (*3*). Our experiments provide the first demonstration that the cyclic AMP content of nervous tissue can change in response to synaptic activity.

Superior cervical sympathetic ganglia were removed from New Zealand white rabbits (weight, 4 to 5 kg) which had been anesthetized with urethane (1000 mg per kilogram of body weight; administered intravenously). Ganglia were carefully decapsulated, mounted on stimulating and recording electrodes in an appropriate chamber, and continuously superfused with oxygenated Locke's solution (*4*). After the response to single shocks was monitored, the ganglia were stimulated through the preganglionic nerve trunk by means of supramaximum shocks at a frequency of 10 per second for various periods of time. Immediately following the stimulus period the ganglia were homogenized in ice-cold 6 percent trichloroacetic acid, and the precipitate was removed by low-speed centrifugation. For comparison, the contralateral ganglion of each pair served as an unstimulated control. The cyclic AMP in the trichloroacetic acid extract was isolated by minor modification of the procedure of Krishna *et al.* (*5*). The amount of cyclic AMP was determined according to its ability to activate a protein kinase purified from bovine heart, by means of the assay method described previously (*3*). Each extract was analyzed in duplicate at two different dilutions, in both the absence and the presence of authentic cyclic AMP added as an internal standard. The protein in the trichloroacetic acid precipitate was determined by the method of Lowry *et al.* (*6*). In some experiments, the cervical vagus, with or without the inclusion of the nodose ganglion, was prepared and studied in a similar manner. The nodose ganglion was studied because it contains nerve cell bodies but not synapses.

The mean concentration of cyclic AMP in a population of 29 unstimulated superior cervical sympathetic ganglia was 17.3 ± 1.3 pmole per milligram of protein. Stimulation of the preganglionic (cervical sympathetic) nerve trunk at a frequency of 10 per second for 30 seconds caused a considerable increase in the cyclic AMP content of the superior cervical ganglion. The concentration of cyclic AMP reached a maximum of about 450 percent of the unstimulated control value within 1 minute of stimulation and remained at this elevated level during the next several minutes of stimulation (Table 1). When experiments with the superior cervical ganglia were carried out at 26°C rather than 33°C, a smaller increase in cyclic AMP, to about 250 percent of control, was observed in response to 2 minutes of preganglionic stimulation (Table 2).

A number of experiments indicate that the increase in cyclic AMP, observed in the superior cervical ganglion as a result of stimulation of the preganglionic nerve trunk, is closely associated with the process of synaptic transmission. Thus, stimulation of these ganglia through the postganglionic (internal carotid) nerve trunk did not result in any significant change in the cyclic AMP content of the ganglia (Table 2). Stimulation of the vagus nerve did not cause any significant increase in the concentration of cyclic AMP either of the nerve itself or of the nodose ganglion (Table 2) under conditions of stimulation that caused a severalfold elevation of cyclic AMP in the superior cervical ganglia. Moreover, the concentration of cyclic AMP in the vagus nerve showed no measurable change even when the nerve was stimulated at a frequency of 50 per second for periods varying between 0.3 and 2.5 minutes. These are conditions that are known to cause large changes in the energy metabolism of the vagus nerve (*7*). The absence of any significant change in the concentration of cyclic AMP upon stimulation of the vagus nerve, stimulation of the nodose ganglion, or postganglionic stimulation of the superior cervical ganglion indicates that the increase in cyclic AMP observed upon preganglionic stimulation of the superior cervical ganglion is indeed associated with the process of synaptic transmission.

It is important to know whether this increase in cyclic AMP, associated with synaptic activity, occurs in the nerve endings of the preganglionic cholinergic nerve fibers or in cells innervated by these nerve endings. In order to distinguish between these two possibilities, we studied the effect of hexamethonium and atropine on the increase in cyclic AMP normally observed in response to preganglionic stimulation. Hexamethonium and atropine are competitive antagonists of the action of acetylcholine on the postsynaptic acetylcholine receptors. It is generally accepted that, in the presence of these antagonists, the preganglionic nerve endings

secrete acetylcholine in a normal fashion, but the postsynaptic electrical response is inhibited. When isolated superior cervical ganglia were superfused for 30 minutes with Locke's solution containing 1 mM hexamethonium chloride plus 0.1 mM atropine sulfate, which nearly abolished the postsynaptic electrical response, the increase in cyclic AMP was also almost abolished (Table 2). When lower doses of these antagonists and longer durations of stimulation were used, so that there was less inhibition of the postsynaptic electrical response, there was also a smaller degree of inhibition of the increase in cyclic AMP that occurred in response to preganglionic stimulation. These results indicate that the increase in the concentration of cyclic AMP associated with synaptic transmission occurred in cells innervated by the preganglionic nerve endings. At most, only a small part of the increase in cyclic AMP could have occurred in the presynaptic terminals themselves. This conclusion is consistent with denervation studies (*8*) which suggest that adenyl cyclase and phosphodiesterase are located in postjunctional cells and is also consistent with recent cytochemical studies (*9*) which demonstrate the localization of phosphodiesterase activity postsynaptically in the vicinity of the synaptic membrane.

It will be important to identify the postsynaptic cells responsible for the increase in cyclic AMP. There are two types of cells in the superior cervical ganglion which appear to be innervated by the preganglionic nerve fibers. Thus, besides the postganglionic neurons, there are present small intensely fluorescent (SIF) cells that also receive preganglionic innervation. There is evidence that these SIF cells function as internuncials to produce the slow postsynaptic potential changes observed in the postganglionic neurons following synaptic transmission: the SIF cells are not only innervated by the preganglionic nerve endings, but they also closely abut and synapse with the soma and dendrites of postganglionic neurons (*10*); the SIF cells contain high concentrations of dopamine (*11*); and exogenous dopamine is known to produce changes in postsynaptic potential similar to those observed after synaptic activity in sympathetic ganglia (*12*). Thus, the slow changes in postsynaptic potential observed after synaptic activity may result from dopamine, released from the internuncial cell, acting upon the postganglionic neurons (*12*). It is well established that concentrations of cyclic AMP in brain slices are increased by certain biogenic amines (*13*). On the other hand, there is no evidence that acetylcholine causes an increase in cyclic AMP content of nervous tissue. It is, therefore, worth considering the possibility that the increase in cyclic AMP, observed during synaptic activity, occurs largely in the postganglionic neurons and is the result of the action of dopamine, secreted from the internuncial cells, on adenyl cyclase in the postganglionic neurons. Recent observations in our laboratory are consistent with such a scheme. Thus, it has been found that low concentrations of dopamine cause an increased accumulation of cyclic AMP in isolated slices of bovine superior cervical ganglia (*14*). Moreover, it seems possible that the postsynaptic hyperpolarization observed in sympathetic ganglia is caused by cyclic AMP which, as demonstrated in the present study, accumulates as a result of synaptic activity. In support of this notion, exogenous norepinephrine causes a hyperpolarization of the cerebellar Purkinje cell membrane, and this effect can be mimicked by cyclic AMP (*15*). It seems quite possible, as has been suggested previously (*16*), that cyclic AMP might produce long-lasting physiological effects on synaptic membranes through a mechanism comprised of the activation of a protein kinase (*17*) and the consequent catalysis of the phosphorylation of a protein constituent of the synaptic membrane. We have, in fact, been able to demonstrate high endogenous levels both of a cyclic AMP–dependent protein kinase (*18*) and of a substrate (*19*) for the protein kinase in fractions rich in synaptic membranes isolated from rat cerebral cortex.

In summary, our working hypothesis, which is consistent with the experimental data so far available, is that dopamine-containing internuncial cells, when activated by acetylcholine released from preganglionic nerve endings, secrete dopamine; the dopamine activates a dopamine-sensitive adenyl cyclase in the postganglionic neurons leading to the formation of cyclic AMP; and the newly formed cyclic AMP, in turn, causes the slow hyperpolarization of these ganglion cells, with consequent modification of their responsiveness to activity in the preganglionic nerve fibers. The present data indicate that adrenergic modulation of cholinergic transmission in sympathetic ganglia may occur through the mediation of cyclic AMP. Such an integrative role for cyclic AMP in ganglia suggests one important generalized function for this nucleotide in nervous tissue: cyclic AMP may provide a molecular basis for integrative actions within the nervous system.

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and the amounts of ^3H and ^{14}C in the isolated sample were counted simultaneously in a Packard Tricarb liquid scintillation system. The amount of radioactivity present in the cyclic AMP of the original samples was calculated per milligram of protein with the use of the recovery of tritiated cyclic AMP to correct each sample for loss during the isolation procedure. The data were corrected by subtracting the amount of radioactive cyclic AMP present in prelabeled but nonincubated tissue.

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Proportional Release of Norepinephrine and Dopamine-β-Hydroxylase from Sympathetic Nerves

Abstract. *Dopamine-β-hydroxylase (DBH), the enzyme that catalyzes the conversion of dopamine to norepinephrine, is localized in the vesicles containing catecholamine in sympathetic nerves. This enzyme is released with norepinephrine when the nerves to the guinea pig vas deferens are stimulated in vitro, and the amount of enzyme discharged increases as the length of stimulation periods increases. The amount of DBH released is proportional to the amount of norepinephrine released, and the ratio of norepinephrine to DBH discharged into the incubation medium is similar to that in the soluble portion of the contents of the synaptic vesicles from the vas deferens. These data are compatible with the release of the neurotransmitter norepinephrine and DBH from sympathetic nerves by a process of exocytosis.*

Norepinephrine is stored in sympathetic nerve terminals within vesicular structures and is released in response to neural stimulation (*1*). The mechanism by which this neurotransmitter is released is not known. Norepinephrine might be liberated from nerves by a process of exocytosis, analogous to the mechanism of release of catecholamines from the adrenal medulla. In this release, the chromaffin granule discharges the soluble portion of its contents to the exterior of the cell presumably through an opening in the cell membrane (*2*). One way to test for a mechanism of release involving exocytosis is to determine whether other soluble molecules in the storage vesicle, especially large molecules, are discharged with norepinephrine in response to stimulation.

Dopamine-β-hydroxylase (DBH), the enzyme that catalyzes the conversion of dopamine to norepinephrine (*3*), is localized in the vesicles storing catecholamine, both in the adrenal medulla (*4*) and in sympathetic nerves (*5*). This enzyme is released with catecholamines when the isolated perfused adrenal gland is stimulated with acetylcholine (*6*) and when the sympathetic nerves to the isolated perfused spleen are stimulated electrically (*7*). For the adrenal medulla, the ratio of norepinephrine to DBH released is similar to the ratio in the chromaffin granule, a result that supports exocytosis as the mechanism of release (*6*). When nerves to the spleen were stimulated, however, the ratio of amine to DBH released was 100 times greater than that found in vesicles isolated from the splenic nerve (*7*). These data have raised serious questions about exocytosis as the mechanism of release of norepinephrine from sympathetic nerves (*8*). The development of a sensitive enzymatic assay for DBH activity (*9*) enabled us to study quantitatively the release of DBH with norepinephrine from sympathetic nerves. The results of these experiments are compatible with the coupled release of norepinephrine and DBH from sympathetic nerves by a process of exocytosis.

The animals used were male albino guinea pigs, 500 to 800 g. Vasa deferentia and attached hypogastric nerves were dissected after the animals were killed by a blow on the head. The organs were placed in 10-ml baths containing medium (*10*) aerated with 5 percent CO_2 in O_2, and were maintained at 37°C. The bath fluid was changed four times and was then replaced by fresh medium containing 0.25 percent bovine serum albumin. This fluid was replaced after 10 minutes with 5 ml of medium containing 0.25 percent albumin, and the organ preparations were allowed to equilibrate for 5 minutes before the start of electrical stimulation, 30 seconds per minute for 30 to 90 minutes (5 to 7 volts, 25 hz, 5 msec).

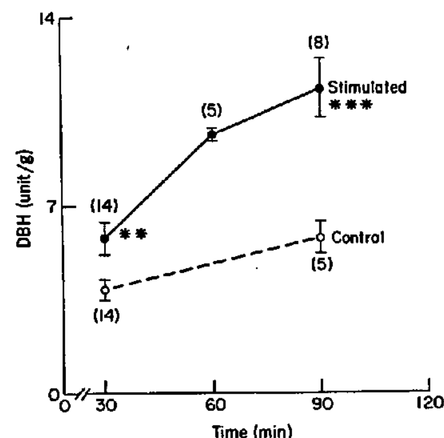

Fig. 1. The release of dopamine-β-hydroxylase after stimulation of hypogastric nerve. Activity of DBH is expressed as nanomoles of octopamine formed from tyramine per gram of tissue per hour. The numbers in parentheses represent the number of vasa deferentia in each group. Symbols are as follows: **, $P < .02$ compared with unstimulated 30-minute control; and ***, $P < .01$ compared with unstimulated 90-minute control.