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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13. The possibility exists that, in our experiments with intact ganglion cells, exogenous dopamine could become concentrated by postganglionic neurons and converted into norepinephrine, and that this newly synthesized norepinephrine, rather than the exogenous dopamine per se, would activate the adenyl cyclase. We consider this to be improbable for several reasons. (i) Low concentrations of dopamine were effective in stimulating the formation of cyclic AMP in homogenates of bovine ganglia; these homogenates were unfortified by the addition of cofactors necessary for the enzymatic conversion of dopamine to norepinephrine. (ii) As described above, experiments with the β-adrenergic antagonist, propranolol, have shown that this agent does not affect the dopamine-mediated increase in cyclic AMP, but does reduce the accumulation of cyclic AMP caused by norepinephrine. (iii) We have found that cocaine (210 μ*M*), which has been shown to block the uptake of dopamine and norepinephrine by various tissues, caused a slight increase in the dopamine-mediated accumulation of cyclic AMP in blocks of bovine ganglia, whereas a decrease would be expected if the intracellular accumulation of dopamine and its conversion to norepinephrine were required. Thus, these data indicate that exogenous dopamine caused the accumulation of cyclic AMP in the ganglion by direct stimulation of an adenyl cyclase sensitive to low concentrations of dopamine.
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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).

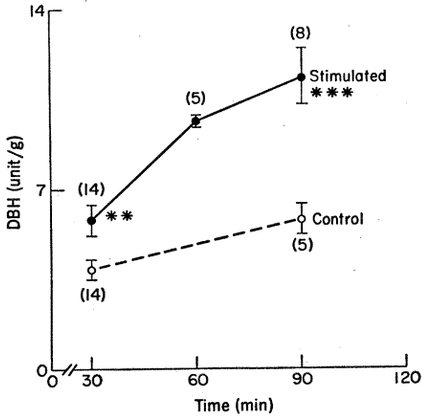

Fig. 1. The release of dopamine-β-hydroxylase after stimulation of hypogastric nerve. Activity of DBH is expressed as nanomoles of octopamine formed from tyramine per gram of tissue per hour. The numbers in parentheses represent the number of vasa deferentia in each group. Symbols are as follows: **,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

Table 1. Ratios of norepinephrine to DBH in tissue and bath fluid. Norepinephrine (NE), DBH activity, and the ratio of NE to DBH are shown for both bath fluid and vas deferens. The incubation medium was collected after 30 minutes of stimulation in the presence of either phenoxybenzamine or desmethylimipramine. The tissue samples were unstimulated vasa deferentia incubated in the presence of the same concentrations of the drug.

Fluid			Tissue		
NE (ng/g)	DBH (unit/g)	Ratio of NE to DBH	NE (ng/g)	Soluble DBH (unit/g)	Ratio of NE to DBH
		*Phenoxybenzamine**			
442 ± 40	8.98 ± .45	49.2 ± 3.4	6163 ± 285	217 ± 16	28.4 ± 3.4
		Desmethylimipramine†			
420 ± 58	7.42 ± .68	56.6 ± 5.6	6660 ± 408	217 ± 16	30.7 ± 1.6

* Fluid samples, 24; tissue samples, 15. † Fluid samples, 10; tissue samples, 10.

Catecholamines were assayed by the trihydroxyindole method after they were isolated on an alumina column (*11*). Activity of DBH was determined by a sensitive enzymatic assay (*9, 12*). In all assays 1 m*M* tyramine was used as substrate. To eliminate the effects of endogenous inhibitors of DBH (*13*) and to obtain optimal DBH activity, both bath fluid and tissue homogenates were assayed in the presence of six different concentrations of $CuSO_4$ ranging from 0 to 32 μM. The most effective concentrations of $CuSO_4$ were 13 μM for bath fluid and 26 μM for a 1 : 200 dilution of a tissue supernatant fraction. The DBH reaction was carried out for 30 minutes for tissue homogenates and for 2 hours for bath fluid. The reaction was linear for 2.5 hours when bath fluid was assayed. Activity of purified bovine adrenal DBH disappeared from the organ baths under the conditions of incubation unless 0.25 percent albumin was added to the bath fluid. The albumin alone had no DBH activity.

Electrical stimulation of the nerves to the guinea pig vas deferens in vitro for 30 minutes resulted in release of DBH into the bath fluid (Fig. 1). The DBH activity discharged into the bath increased when length of the stimulation period was increased up to 90 minutes. The DBH activity in the bath fluid containing unstimulated vasa deferentia also increased with time of incubation, but the difference between stimulated and unstimulated preparations was more than twofold greater after 90 minutes of stimulation than after 30 minutes (Fig. 1).

To measure norepinephrine released into the bath fluid, it was necessary to block the mechanism for catecholamine reuptake by the nerve terminals (*14*). This was accomplished by adding phenoxybenzamine or desmethylimipramine, drugs which block neuronal uptake. When phenoxybenzamine ($3 \times 10^{-5} M$) was added to the bath fluid, the release of norepinephrine and DBH were proportional, with an increased release of norepinephrine being reflected in an increased release of DBH (Fig. 2). The same thing occurred when desmethylimipramine ($10^{-5} M$) was used to block amine reuptake (*15*) (Fig. 2). To eliminate the possibility that the proportional release was due to interference in the DBH assay by norepinephrine or by some other substance released from the vas deferens, samples from an experiment in which phenoxybenzamine was present in the incubation medium were assayed for DBH activity in the presence and absence of tyramine, the substrate for the DBH reaction. No activity was observed in the absence of tyramine.

To compare the ratio of norepinephrine to DBH in tissues with the ratio of these substances after they were released into the bath fluid, stimulated and unstimulated vasa deferentia incubated with either phenoxybenzamine or desmethylimipramine were stripped of connective tissue, frozen on Dry Ice, thawed at 4°C, and homogenized in 100 volumes of water to lyse the vesicles containing catecholamine. The homogenate was centrifuged at 100,000*g* for 1 hour. Activity of DBH and content of norepinephrine were determined in both the supernatant fluid and pellet. About two-thirds of the norepinephrine in the organ was released into the supernatant fluid by this treatment, but only 12 to 16 percent of the DBH activity was found there. The ratios of norepinephrine to DBH in tissue and in the incubation medium after 30 minutes of stimulation are shown in Table 1. Although the ratios are lower in tissue than in bath fluid, they are similar.

In earlier studies, a disproportionately high ratio of norepinephrine to DBH was found in the incubation medium (*7*). Among the possible explanations for this high ratio are the lability of DBH activity at low concentrations in perfusion fluid and the use of splenic nerve axons to establish tissue ratios. The axons were used rather than the spleen, which contains the nerve terminals, because potent endogenous inhibitors of DBH are present in the spleen (*7*). However, the vesicular structures in the axons of sympathetic nerves differ morphologically from those found in nerve terminals, with large rather than small vesicles being present in the axons (*16*). This makes it difficult to extrapolate

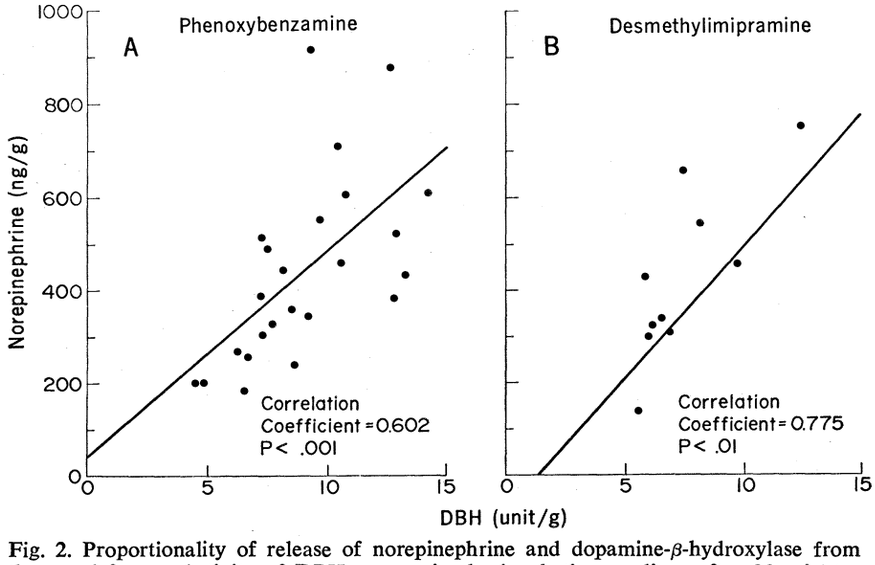

Fig. 2. Proportionality of release of norepinephrine and dopamine-β-hydroxylase from the vas deferens. Activity of DBH present in the incubation medium after 30 minutes of stimulation in the presence of phenoxybenzamine (A) or desmethylimipramine (B) is plotted against norepinephrine released into the bath; DBH activity is expressed as in Fig. 1 and norepinephrine is expressed as nanograms released per gram of tissue.

biochemical data for the nerve terminal from that obtained in the axon.

Dopamine-β-hydroxylase is present in the vesicles in both the adrenal medulla and the sympathetic nerves in bound and soluble forms (*5, 17*). The soluble form is that released by disruption of the vesicle by osmotic shock. The portion of DBH that is most likely to be discharged after stimulation of nerves is the soluble form. Experiments with the adrenal medulla where, depending on the species, 20 to 50 percent of the DBH activity can be liberated from chromaffin granules by osmotic shock, have shown that the soluble portion of the DBH is that which is depleted by stimulation (*17*). The results reported here agree with other reports in which a lower proportion of soluble DBH appears to be present in vesicles from sympathetic nerves than is present in the adrenal gland (*5*).

Since 85 percent of DBH is in bound form and the estimated half-life of the sympathetic nerve vesicle is 3 weeks (*8*), it is likely that the vesicle is reused. The ratio of amine to DBH released from the vas deferens is higher (although close) to that found in tissue. This might result from preferential release of newly synthesized norepinephrine (*14*). Preferential discharge of newly synthesized amine from a population of vesicles that have already liberated some or all of their releasable DBH could explain the apparent increase in release of norepinephrine relative to DBH into the bath fluid. Finally, the assumption that diffusion from the synaptic cleft of norepinephrine, a molecule with a molecular weight of 169, occurs at the same rate as that of DBH, a protein with a molecular weight of 300,000 (*3*), might be incorrect. Unequal diffusion rates would contribute further to the increase in the ratio of amine to enzyme found in the bath fluid.

The similarity of the ratios of catecholamine to DBH in tissue and in the incubation medium, despite the possible sources of error discussed above, and the proportionality of amine to DBH discharged are compatible with the coupled release of the neurotransmitter and the enzyme from sympathetic nerves by a process of exocytosis.

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Analgesia from Electrical Stimulation in the Brainstem of the Rat

Abstract. *Stimulation at several mesencephalic and diencephalic sites abolished responsiveness to intense pain in rats while leaving responsiveness to other sensory modes relatively unaffected. The peripheral field of analgesia was usually restricted to one-half or to one quadrant of the body, and painful stimuli applied outside this field elicited a normal reaction. Analgesia outlasted stimulation by up to 5 minutes. Most electrode placements that produced analgesia also supported self-stimulation. One placement supported self-stimulation only in the presence of pain.*

The management of prolonged pain in man has been a stubborn medical problem because the most effective analgesic drugs carry with them such undesirable side effects as tolerance and dependence. Alternative approaches have long been sought. The approach of making discrete brain lesions has not been consistently successful in alleviating either pain states in man or experimentally evoked pain in animals. This failure to excise pain or to interrupt the pathways responsible for its appreciation and expression seems to indicate that the neural substrate of pain is so diffuse or redundant as to escape focally inflicted brain damage. On the other hand, some hope that pain can be alleviated by a neurosurgical procedure has been offered by a small number of studies reporting pain reduction that accompanies electrical stimulation of discrete brain regions in man as well as lower animals (*1, 2*). The animal work has typically involved effects of small or indeterminate magnitude [however, see (*1*)], and from these reports it has not been sufficiently clear whether the effect was directly on pain perception or was a reflection of broader deficits in sensory, motor, or motivational mechanisms. Clinical attempts to utilize this approach have been understandably few, and the number of brain areas probed quite restricted. We report that analgesia from focal brain stimulation in the rat can be of such magnitude as to render the animal totally unresponsive to pain. We show that such analgesia results from stimulation in a number of subcortical loci, including those not previously tested in man. Evidence is provided that analgesia can occur without apparent accompanying sensory, motor, or motivational deficits.

Bipolar electrodes were implanted bilaterally in various mesencephalic and diencephalic loci in 22 male Sprague-Dawley rats. Electrodes were made of twisted, stainless steel wires (200 μm in diameter) insulated except at the cut cross sections of their