tion of mitochondrial DNA leading to long-term inhibition of mitochondrial biosynthetic processes and perhaps to mitochondrial mutational events may directly contribute to the carcinogenic process.

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16. We thank A. Bhat for technical help and N. Leinwand for helping with the preparation of the manuscript. This research was supported by NIH grant CA-22762.

17 July 1981

Substance P Counteracts Neurotoxin Damage on Norepinephrine Neurons in Rat Brain During Ontogeny

Abstract. *Systemic treatment of newborn rats with the catecholamine neurotoxin 6-hydroxydopamine alters the postnatal development of the central norepinephrine neurons. The changes are permanent and consist of denervation of distant nerve terminal projections (for example, cerebral cortex) and hyperinnervation of terminal areas close to the cell bodies (for example, cerebellum). Intracisternal injection of substance P counteracted both of these alterations. The results indicate that substance P may prevent degeneration of damaged norepinephrine neurons during ontogeny or may have a regrowth stimulatory action on these cells. Substance P might prove of use in the prevention or reduction of other types of neurodegenerative disease.*

The monoamine-containing neurons in the central nervous system (CNS) appear very early during development in many species including man. In rat the cell bodies are already formed on gestation days 12 to 14, and the axonal pathways are fully developed at birth. The postnatal development of central monoamine neurons consists mainly of a proliferation of the nerve terminals in the regions that they innervate (*1*). Systemic administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) in the neonatal stage markedly alters the postnatal development of the central norepinephrine (NE) neurons, in particular those of the locus coeruleus, which has extensive collaterals and innervates many brain regions. This treatment thus leads to permanent and pronounced denervations of distant NE terminal projections (for example, in the cerebral cortex and spinal cord), whereas projections close to the cell bodies (for example, in the cerebellum and pons medulla) increase, leading to a hyperinnervation (*2*, *3*).

The consequences of neonatal administration of 6-OHDA on the central NE neurons are mainly related to a "pruning effect" (*4*). The data point to a rigid growth pattern of these neurons, especially with respect to the development of the number of NE nerve terminals, independent of the regions they innervate. The development of axonal arborizations and terminals may therefore be regulated mainly at the level of the perikarya, for example, by way of their synaptic inputs. The NE perikarya of the locus coeruleus receive various types of inputs, among others, nerve terminals containing the peptide substance P (SP) (*5*), which has an excitatory effect (*6*). We now report that neonatal administration of SP can modify the altered postnatal development of central NE neurons induced by neonatal administration of 6-OHDA.

Newborn rats (Sprague-Dawley) were treated with 6-OHDA (100 mg/kg, subcutaneously) or substance P (2×5 to 50 μg in 10 μl of solvent, intracisternally, at intervals of 20 hours). Some animals received both 6-OHDA and SP, in which case the first dose of SP was administered 4 to 6 hours after the 6-OHDA. Controls received identical injections of the solvent. The rats were raised to the age of 3 to 6 weeks, when they were killed and samples were taken from the CNS for neurochemical and histochemical analyses. Endogenous NE was assayed according to the method of Keller *et al.* (*7*) and the uptake of ^{3}H-labeled NE was measured in vitro as described by Lidbrink and Jonsson (*8*). Fluorescence histochemical demonstration of catecholamines was performed according to the method of Lorén *et al.* (*9*).

In agreement with previous studies (*2*, *3*) and compatible with the pruning effect concept, the neonatal administration of 6-OHDA depleted endogenous NE in the frontal cortex (−90 percent), the occipital cortex (−99 percent), and the spinal cord (−97 percent), whereas this treatment more than doubled the NE concentrations in the cerebellum and the pons medulla (Table 1). Administration of SP (2×50 μg) alone did not significantly alter NE except in the pons medulla where a small (+16 percent) but significant ($P < .05$) increase was found. Intracisternal injection of SP (2×50 μg) in animals previously treated with 6-OHDA significantly counteracted the NE depleting effect induced by 6-OHDA. This effect of SP was most pronounced in the frontal cortex. Substance P also counteracted the 6-OHDA–induced increases of NE, both in the pons medulla and cerebellum.

We also tested the effect of various doses of SP on the altered NE concentrations produced by 6-OHDA in the frontal cortex. The results demonstrated a clear dose-response relationship; 2×5 μg of SP had no effect (17 ± 9.4 percent of control) on the NE reduction produced by 6-OHDA (15 ± 4.1 percent of control), whereas both 2×10 μg and 2×25 μg of SP had clear counteracting effects, the NE levels being 35 ± 9.6 and 54 ± 6.3 percent, respectively, of control (191 ± 24 ng/g; $N = 4$ and 5, respectively). The effectiveness of 2×25 μg of SP was approximately similar to that of 2×50 μg (see Table 1).

In agreement with the results of the NE assay, 2×50 μg of SP had a normal-

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Table 1. Effects of neonatal administration of 6-OHDA (2 × 50 mg/kg, subcutaneously) and SP (2 × 50 μg, intracisternally) on the NE concentration in various brain regions of 6-week-old rats. The data are presented as nanograms of NE per gram of tissue, wet weight.

Region	Control	SP	6-OHDA	6-OHDA plus SP
Frontal cortex	332 ± 9.0	334 ± 17	35 ± 10	157 ± 29*
Occipital cortex	278 ± 14	262 ± 23	1.8 ± 0.9	18 ± 5.1*
Cerebellum	191 ± 7.6	211 ± 13	443 ± 9.4	316 ± 7.3†
Pons medulla	841 ± 16	977 ± 34	1968 ± 122	1435 ± 124*
Spinal cord	493 ± 65	429 ± 15	13 ± 2.1	37 ± 6.9‡

*$.01 > P > .001$. †$P < .001$ (Student's t-test, $N = 6$ or 7). ‡$.05 > P > .01$.

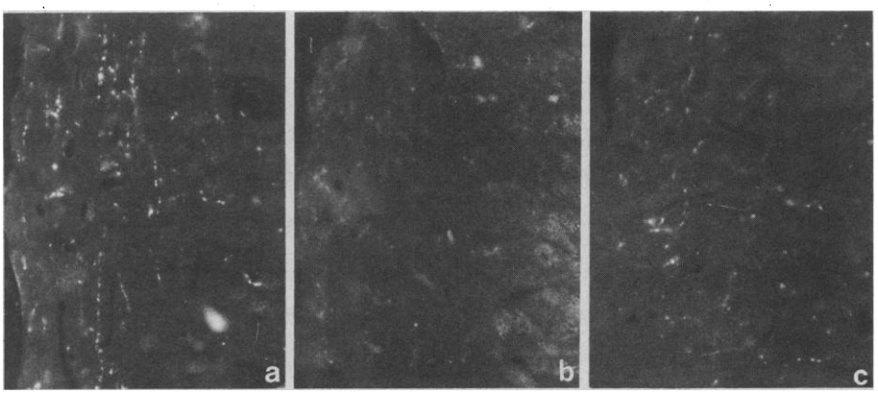

Fig. 1. Fluorescence histochemical demonstration of NE nerve terminals in superficial layers of the rat frontal cortex (×370). The animals were treated at birth and killed at the age of 3 weeks. (a) Control. (b) 6-OHDA; there is a marked NE denervation and only single terminals can be seen. (c) 6-OHDA plus SP; more fluorescent NE nerve terminals can be seen compared with 6-OHDA treatment alone.

izing effect on the 6-OHDA–induced reduction in ^{3}H-labeled NE uptake in slices from the frontal cortex in vitro. The 6-OHDA treatment alone reduced the uptake to 19 ± 10 percent of control (3.3 ± 0.56 nCi per slice; $N = 8$), whereas this value was 53 ± 12 percent after the administration of 6-OHDA followed by SP. Treatment with SP alone did not significantly alter the labeled NE uptake compared to control. Furthermore, the 6-OHDA–induced increase (209 ± 13 percent of control) in labeled NE uptake in slices from the cerebellum was clearly reduced by subsequent SP treatment (147 ± 14 percent). Treatment with SP alone did not cause any change compared to control (1.5 ± 0.14 nCi per slice; $N = 8$). Results obtained from fluorescence histochemical analysis of brains after neonatal administration of 6-OHDA alone or with SP were in agreement with the neurochemical results. The 6-OHDA–induced NE denervation in the cerebral cortex was thus clearly counteracted by SP (Fig. 1). The increase in NE nerve density in the cerebellum produced by 6-OHDA was also counteracted by treatment with SP.

These results show that SP has a sparing effect in regions where 6-OHDA induces an otherwise permanent denervation of NE nerve terminals. Whether the sparing effect occurs at the axonal level promoting regenerative sprouting or collateral sprouting remains to be elucidated. If one assumes that the altered development of NE neurons produced by 6-OHDA is related to a pruning effect (*4*), it is conceivable that the counteracting effect of SP on the hyperinnervation in the cerebellum and pons medulla is a consequence of the sparing effect on the NE denervations in remote areas. Neonatal administration of SP alone did not affect postnatal development of catecholamine neurons, although a small increase in NE was noted in the pons medulla. We do not know whether this increase was due to a change in NE nerve density or to a change in steady-state concentrations of the transmitter. Although our data suggest that SP alone did not cause any substantial effects on the postnatal development of the central NE neurons from a structural viewpoint, the possibility that substance P altered the functional state of the NE neurons cannot be excluded.

It is unlikely that SP interferes with the neurotoxicity of 6-OHDA in the cerebral cortex and spinal cord, because we have found no evidence in vitro of SP affecting the NE uptake mechanism of 6-OHDA on which the neurotoxic action of 6-OHDA depends (*10*). Moreover, SP was administered after the cytotoxic action of 6-OHDA on NE nerve terminals was largely completed (*3, 10, 11*). It is possible that SP acts as a neurotrophic agent that stimulates the growth of damaged NE neurons; there is already evidence that SP can stimulate neurite outgrowth and be of trophic importance (*12*). Another possibility could be that SP, by exciting the locus coeruleus neurons (*6*) and activating their metabolism, reduces their degeneration or stimulates their regrowth. It is of interest in this context that electrical stimulation produces prolonged and sustained improvement in bladder function after spinal cord injury (*13*). This therapeutic effect of electrical stimulation may be related to a nerve growth stimulatory effect that promotes functional recovery. That changes in electrical activity modify the extent of the 6-OHDA–induced alteration of the postnatal development of the locus coeruleus system, is in agreement with recent observations that nicotine, which—like SP—has an excitatory effect on locus coeruleus neurons (*14*), also has a counteracting effect, whereas morphine, which has an inhibitory effect (*15*), potentiates the 6-OHDA–induced NE denervations and hyperinnervations (*16*).

Our results show that SP can counteract the denervations and hyperinnervations induced by 6-OHDA in the postnatal development of central NE neurons. The exact mode of action of this SP effect is unknown, although it might be related to prevention of degeneration or stimulation of regrowth, which in turn might be associated with the excitatory effect of SP on the locus coeruleus neurons. These results thus suggest that SP might have clinical applications, for example, in preventing or diminishing neurodegenerative processes in the brain that may be spontaneous (due to aging), traumatic, or neurotoxic in origin.

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17. This research has been supported by grants from the Swedish Medical Research Council (04X-02295), Expressens Prenatalforskningsnämnd, and Bergvall's and Jeansson's Foundations.

28 April 1981; revised 3 August 1981

Pulmonary Blood Plasma Filtration in Reptiles: A "Wet" Vertebrate Lung?

Abstract. *The net loss of plasma from blood into tissues within the ventilated reptile lung is 10 to 20 times greater than that in mammalian lungs. When blood flow is reduced during breathholding by reptiles, the plasma loss stops, and a net reabsorption of fluid from the tissues occurs. Fluid movement dynamics and the relative "dryness" of the lung of reptiles and mammals thus differ in several important respects and reflect the more variable cardiovascular performance of reptiles.*

Fluids continually move in both directions through the walls of blood capillaries. The net hydrostatic pressure (blood pressure) forcing plasma from capillaries is nearly completely opposed by the net colloid osmotic pressure drawing tissue fluid back in, so that net blood plasma loss is very low. In mammalian lungs, only 1 to 4 percent of the blood fluids is lost to the surrounding tissues (*1*), this fluid being effectively drained away by lymphatic vessels. The alveoli (gas sacs) within mammalian lungs thus remain comparatively dry, greatly facilitating diffusion of O_2 and CO_2.

Our current concept of lung fluid balance is based almost entirely on the "dry" mammalian lung, which is constantly perfused at a mean arterial blood pressure of 10 to 15 mmHg (*2*). In reptiles, however, mean pulmonary arterial pressures may be up to five times higher than those in mammals (*3, 4*), because the typical reptilian heart, with its single ventricular pump, produces very similar pulmonary and systemic pressures (*4*). In view of the elevated pulmonary arterial pressure in reptiles, it is paradoxical that the colloid osmotic pressure of reptilian plasma is lower than that of mammals (*5*), and would seem to be far too low to keep the lung dry by effectively counterbalancing plasma filtration from pulmonary capillaries.

The lungs of reptiles thus appear to be perfused under physiological conditions that are incompatible with our current mammalian-based concepts of tissue fluid regulation. This study, in which measurements were made of the net fluid movements between the vascular and nonvascular spaces of the lung of an ectothermic vertebrate, raises the question of whether a dry lung is a trait of all air-breathing vertebrates.

The turtle *Chrysemys (Pseudemys) scripta* was chosen because of its high pulmonary arterial blood pressure (*4*) and the ease of access to its lungs and blood vessels. Extracellular fluid in the lung was assumed to be in one of two compartments—vascular (within blood vessels) or nonvascular (extracellular tissue spaces, alveoli, and lymphatic vessels). Instantaneous net flow of fluid between these two lung compartments was measured with an electromagnetic flow probe and a modification of an indicator dilution method for measuring capillary permeability (*6*). Instead of using an artificial indicator, I based calculations on red blood cell (RBC) concentrations in simultaneously drawn samples of pulmonary arterial and pulmonary venous blood (*7*). For each of eight turtles, four to six paired blood samples (100 μl) for RBC counts were taken within a 24-hour period, during which pulmonary arterial blood pressure and left pulmonary artery flow were monitored continuously (*8*). Values, reported as means ± standard deviation ($N = 40$), for heart rate (24 ± 6 beats per minute), blood pressure (17 ± 6 mmHg) (*9*), and minute blood flow to the left lung (7.38 ± 4.82 ml/kg) were in excellent agreement with reported values (*3, 4*), as were cardiovascular events that occurred during intermittent lung ventilation (Fig. 1).

The concentration of RBC's in pulmonary venous blood was often 10 to 40 percent higher than that in pulmonary arterial blood, a disparity many times greater than is found in rabbit lung vessels (*10*). However, variation was common between pairs of samples, even when taken only 2 minutes apart. These paired RBC counts, as well as lung blood flow at the time of sampling, were used to calculate the net flow and direction of plasma movement in the lung. There was a significant correlation ($r = .78$; $P < .001$) between the rate of plasma filtration and pulmonary blood flow (Fig. 2). At blood flows of 12 to 14 ml/kg-min evident during lung ventilation, 20 to 30 percent of the fluid entering the lung remained behind in the nonvascular lung tissues. However, when blood flow fell to only 2 to 4 ml/kg-min during breathholding, plasma filtration stopped or even reversed, causing reabsorption of fluid from pulmonary nonvascular spaces. There was no significant relation between lung filtration or reabsorption and mean pulmonary arterial pressure, which fluctuated during intermittent breathing (Fig. 1).

The most striking difference between pulmonary filtration dynamics in mammals and reptiles is the apparent "leakiness" of the turtle pulmonary capillaries. When lung blood flow is high during ventilation, the volume of plasma filtrate is approximately 10 to 20 times greater than that in mammalian lungs (*1*). A contributory factor may be the high pulmonary arterial blood pressure of *Chrysemys*, which exceeded mammalian pulmonary arterial pressures by 20 to 40 percent. Capillary blood pressure (which remains to be measured) will be lower, but some proportion of the elevated arterial pressure of *Chrysemys* is almost certainly transmitted to the lung capillaries. Since blood colloid osmotic pressure of turtles is lower than that in mammals (*5*),

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