Supraoptic Neurosecretory Cells: Adrenergic and Cholinergic Sensitivity

Abstract. *Adrenergic and cholinergic agonists and antagonists were applied microelectrophoretically to over 700 neurons in the cat supraoptic nucleus, 20 percent of which were antidromically identified as neurosecretory cells. Norepinephrine uniformly depressed all sensitive cells. Acetylcholine caused both muscarinic depression and nicotinic excitation which were antagonized by atropine and dihydro-β-erythroidine, respectively. These results support the hypothesis that norepinephrine and acetylcholine are directly involved in controlling the release of antidiuretic hormone.*

Antidiuretic hormone (ADH) is synthesized in supraoptic neurosecretory cell bodies and transported along their axons to the posterior pituitary where it is released into capillaries (*1*). Evidence has been presented (*2*) to support the hypothesis that release of ADH is related to an increase in the discharge rate of these neurons (*3*). Both adrenergic (*4*) and cholinergic (*5*) mechanisms have been implicated in the release of ADH. In general, the experiments have suggested adrenergic inhibition and cholinergic stimulation of ADH release; but this research has led to variable results, depending on the drug, preparation, dosage, route of administration, and assay of antidiuretic hormone used. Furthermore, previous research could not exclude pharmacologic effects on populations of cells, other than neurosecretory cells which might indirectly influence ADH release. These factors have prompted us to examine the responsiveness of individual supraoptic neurons to the direct microelectrophoretic application of adrenergic and cholinergic agonists and antagonists. We now report consistent adrenergic and cholinergic responses of antidromically identified neurosecretory cells and other neurons in the supraoptic nucleus (*5a*).

Forty cats, decerebrated or anesthetized with urethane (1.2 g per kilogram of weight) were placed supine in the stereotaxic apparatus and the pituitary gland and optic chiasm were exposed through the roof of the mouth. A metal, bipolar stimulating electrode was placed under direct vision in the posterior lobe of the pituitary for the purpose of antidromically identifying supraoptic neurosecretory cells by electrical stimulation of the neurohypophysis (*6*). Five-barrel micropipettes, prepared according to methods previously described (*7*), were used for unit recording and microelectrophoretic application of pharmacologic agents (*8*).

Norepinephrine (NE) depressed the activity of 90 percent of the neurosecretory cells studied (Fig. 1A); the rest were unresponsive. Similarly, all other responsive supraoptic neurons were depressed by norepinephrine (Table 1). The beta-adrenergic blocking agent MJ-1999 (*9*) antagonized the depression caused by norepinephrine in five of seven cells tested (Fig. 1B).

In contrast to the uniform norepinephrine depression, responsive neurosecretory cells exhibited either excitation or depression after application of acetylcholine (ACh) (Fig. 1A). Eighty-four percent of responsive neurosecretory cells were depressed by ACh; the rest were excited. A slightly smaller ratio of depressions to excitations was found in the uninvaded population (Table 1). Norepinephrine depression and either ACh depression or excitation could be demonstrated in the same neurosecretory cell. The dual nature of the cholinergic response suggested that two types of receptors might be present on neurosecretory cells. Therefore, muscarinic and nicotinic agonists were applied to these cells. Acetyl-β-methylcholine (MeCh) and carbachol (carbaminoylcholine), cholinergic agonists with both muscarinic and nictotinic

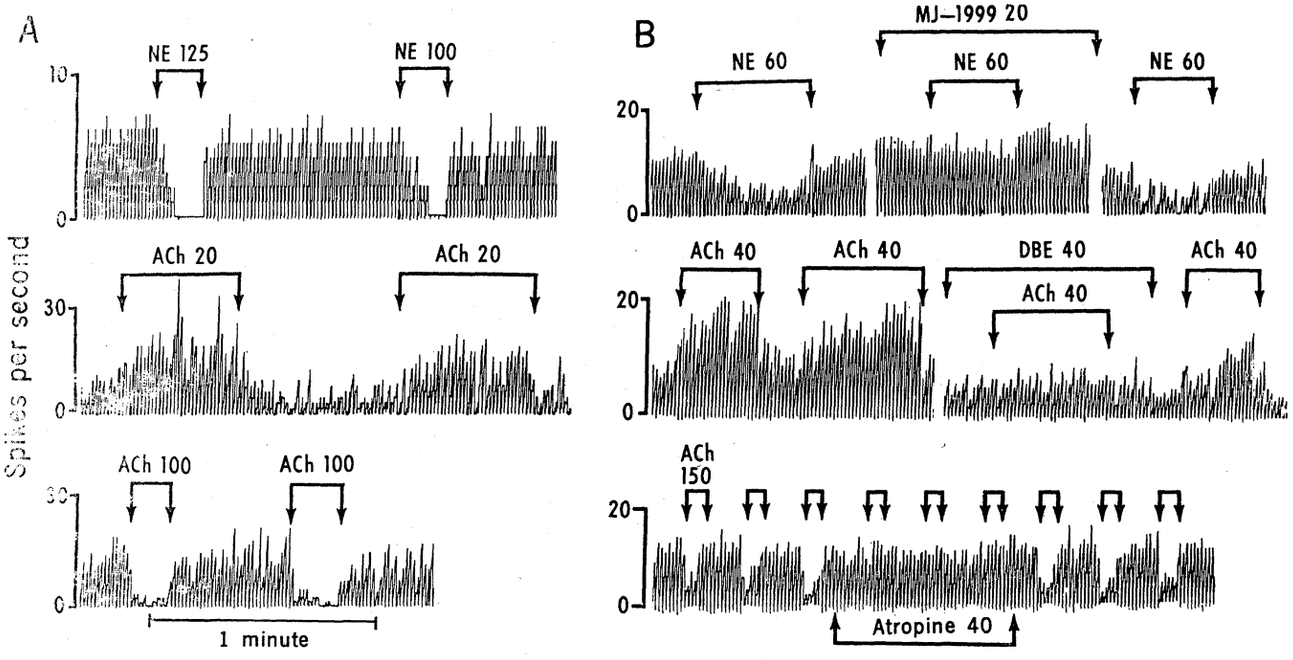

Fig. 1. Responses of neurons in the supraoptic nucleus to norepinephrine and acetylcholine. (A) Norepinephrine depression and ACh excitation and depression of activity (1-second integrations) in three different, antidromically identified neurosecretory cells. (B) Blocks of these responses in uninvaded supraoptic neurons: (top) blockade of NE depression by the beta-adrenergic blocking agent MJ-1999; (middle) antagonism of ACh excitation by dihydro-β-erythroidine, an antagonist of nicotine; and (bottom) block of ACh depression by atropine, an antagonist of muscarine. Numbers next to drug abbreviations refer to current in nanoamperes.

properties in other systems (*10*), depressed all 70 of the 110 neurons tested. Nicotine excited 12 of the 13 responsive neurosecretory cells and 66 percent of the 58 sensitive, uninvaded neurons. Both nicotine excitation and MeCh depression could be elicited in the same cell. These findings suggested the existence of two populations of functionally distinct cholinoceptive sites on neurosecretory cells: activation of muscarinic receptors producing depression of activity and activation of nicotinic receptors producing excitation.

To further test this hypothesis we used muscarinic and nicotinic antagonists. Application of atropine, an antagonist of muscarine (*11*), blocked both ACh (Fig. 1B) and carbachol or MeCh depressions in 70 percent of the 25 cells studied, but did not block either ACh or nicotine excitations in ten neurons tested. Microelectrophoresis of dihydro-β-erythroidine, an antagonist of nicotine (*12*), blocked both ACh (Fig. 1B) and nicotine excitations in 80 percent of the 15 cells examined, but failed to antagonize either ACh or carbachol depression on 18 other neurons. The antagonism of muscarinic depressions and nicotinic excitations by only the appropriate antagonist further supports the notion of two functionally distinct cholinergic receptors on neurosecretory cells.

Previous microelectrophoretic research on the central nervous system (*13*) and hypothalamus (*14*) has shown a widespread sensitivity to norepinephrine and acetylcholine. In general, norepinephrine depresses (*15*) more often than excites (*16*) central neurons. Cholinomimetics are usually excitatory, causing predominantly muscarinic excitation of cortical (*17*) and thalamic (*18*) neurons and primarily nicotinic excitation of Renshaw cells (*19*). Our results in the supraoptic nucleus reveal a high degree of uniform sensitivity to norepinephrine, comparable to that reported for cerebellar Purkinje cells (*20*). The demonstration of cholinoceptive sites with opposing functions complements the recent work of Wachtel and Kandel (*21*) on invertebrate central neurons and provides evidence that the nature of the response to ACh is determined by distinctly different receptors on the postsynaptic membrane of the neurosecretory cell.

The presence of norepinephrine nerve terminals ending on supraoptic neurons (*22*) and of ACh with its appropriate enzyme systems (*23*) is well documented. Treatment with reserpine results in the disappearance of supraoptic norepinephrine fluorescence (*22*) and reportedly stimulates ADH secretion (*24*). Dehydration increases supraoptic acetylcholinesterase content (*25*) and ADH activity (*26*). This evidence coupled with other indirect reports from the pharmacologic literature (*4*, *5*) and the present demonstration of a consistent sensitivity to the direct application of NE and ACh provides strong support for the hypothesis that these substances are synaptic transmitters which modulate supraoptic neurosecretory activity (*27*). We propose that norepinephrine is inhibitory and ACh both inhibitory (muscarinic) and excitatory (nicotinic) at synapses ending on the neurosecretory cell.

Table 1. Responses of neurons in the supraoptic nucleus to ACh and NE.

Response	Cells (No.)	
	Neurosecretory	Uninvaded
	Norepinephrine	
Depressed	38	171
No response	4	74
	Acetylcholine	
Depressed	30	91
Excited	6	36
No response	17	97

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Olfactory Bulb Removal Eliminates Maternal Behavior in the Mouse

Abstract. *Olfactory bulb removal eliminated maternal behavior in lactating and in virgin mice. The results are in contrast to the generally accepted concept of multisensory control of mammalian maternal behavior.*

Data derived principally from the rat have led to the traditional view that mammalian maternal and sexual behaviors are under multisensory control (*1*)—that is, no one sensory system is essential for the exhibition of those behaviors. This concept has recently been challenged by a report showing that olfactory bulb removal eliminates sexual behavior in the hamster (*2*). The role of the specific sensory systems in maternal behavior has not, however, been carefully examined in mammalian species other than the rat. The observations reported here clearly demonstrate that the integrity of the olfactory system is a prerequisite for maternal behavior in the mouse.

In the first experiment, 20 albino Rockland-Swiss (R-Swiss/Z) and 20 C57BL/10Gin multiparous, pregnant mice were divided into two equal groups, one of which sustained removal of the olfactory bulbs while the other underwent sham operations (*3*). Bilateral bulbectomy, performed under ether anesthesia, consisted of drilling a 2-mm hole in the skull approximately 5.0 mm anterior to the bregma and removing the tissue by suction. Sham operations consisted of everything except tissue removal. After surgery, each animal was individually housed in an 11 by 7 by 5 inch (approximately 28 by 18 by 13 cm) fiber-glass cage, the floor of which was covered with wood shavings. Animals had free access to food and water and were kept on a 12-hour light-dark cycle.

At the time of parturition, which occurred 1 to 11 days after surgery, the general bodily condition of the young and the behavior of the adult were recorded every morning and afternoon. An adult was scored as exhibiting maternal behavior if it was seen to be licking or carrying a pup, to be in a nursing posture, or to be constructing a nest. Observations were continued to weaning at 21 days or until the death of the pups. At the termination of testing, the adult animals were killed and their brains were macroscopically examined to determine the extent of the ablation. Mammary tissue was also examined.

Of 20 mice that underwent bulbectomy, 18 displayed no maternal behavior, whereas 19 of 20 sham-operated animals showed maternal behavior until weaning. This difference is statistically significant ($P < .001$). The results are summarized in Table 1.

Of the bulbectomized mice, 16 ate their young; they ate either all or part of their litters, which averaged eight pups. Cannibalism typically occurred within 12 hours of parturition, although two animals ate their young on the second postpartum day. Two ablated mice ate none of their litter but showed no maternal behavior toward any of their pups. Behaviors such as eating of the placenta and cleaning of the young, which occur at parturition, were exhibited by all animals. However, in all but two cases, bulbectomized animals were never seen to build nests or to engage in any form of maternal behavior toward the pups that were eventually eaten or to the pups that were left alone until death. Pups that were ignored died within 2 days of birth. Two adult females did place their young together and nurse the pups occasionally for about 4 hours before eating them.

Inspection of the brains revealed that the two mice in the bulbectomized group that displayed maternal behavior until weaning sustained only unilateral bulbectomies, whereas bilateral bulbectomies were sustained by the remaining subjects. Mammary tissue of the experimental subjects did not differ from that of the sham operates in terms of extent or ability to extrude milk.

These data indicate that removal of the olfactory bulbs leads to an elimination of maternal behavior and, in most instances, to an initiation of cannibalism. Furthermore, both olfactory bulbs must be removed for the effect to be seen.

Table 1. Behavior exhibited toward pups by C57BL/10Gin and R-Swiss/Z mice that either sustained olfactory bulb removal or underwent sham operations. Surgery was performed at various times throughout pregnancy.

Treatment	Ate all pups	Ate some pups; ignored the remainder	Ignored all pups	Normal maternal behavior
R-Swiss/Z mice				
Bulbectomy ($N = 10$)	7	2	0	1*
Sham ($N = 10$)	0	0	0	10
C57BL/10Gin mice				
Bulbectomy ($N = 10$)	4	3	2	1*
Sham ($N = 10$)	1	0	0	9

* Sustained incomplete bilateral bulbectomies.

Table 2. Behavior exhibited toward pups by R-Swiss/Z mice that either sustained olfactory bulb removal or underwent sham operations. Surgery was performed on day 12 of pregnancy (experiment 2) and in virgin mice (experiment 3).

Treatment	Ate all pups	Ate some pups; ignored the remainder	Ignored all pups	Normal material behavior
Experiment 2				
Bulbectomy ($N = 8$)	7	1	0	0
Sham ($N = 8$)	0	0	0	8
Experiment 3				
Bulbectomy ($N = 20$)	14	1	2	3*
Sham ($N = 21$)	1	0	1	19

* Sustained incomplete bilateral bulbectomies.