in the fetus were able to respond to this stimulus by development in the male direction as seminal vesicles. A heritable form of male pseudohermaphroditism, not due to sex chromosome mosaicism, has been reported in rats; they secrete low levels of testosterone (*20*). The presence of a vagina in these pseudohermaphrodite rats as well as in an allophenic mouse such as case C could result from a subnormal amount of androgen in fetal stages.

From the fact that XO mice are females, it appears that the Y chromosome of the mouse is male-determining (*21*). Inasmuch as XX cells can develop into seminal vesicles and secrete SVP in the absence of any intracellular Y-linked factors, it follows that histogenesis of the vesicles and function of the *Svp* locus in the XY cells of normal males may also be independent of expression of male-determining factors on the Y in those cells.

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References and Notes

1. B. Mintz, *Amer. Zool.* **2**, 432 (1962); *Science* **148**, 1232 (1965).
2. ———, in *Methods in Mammalian Embryology*, J. C. Daniel, Jr., Ed. (Freeman, San Francisco, 1971), p. 186.
3. ———, *Symp. Int. Soc. Biol.* **9**, 15 (1970).
4. ———, *Proc. Nat. Acad. Sci. U.S.* **58**, 344 (1967).
5. ———, *Genetics* **61** (Suppl.), 41 (1969).
6. B. Mintz and S. Sanyal, *ibid.* **64** (Suppl.), 43, (1970).
7. H. Condamine, R. P. Custer, B. Mintz, *Proc. Nat Acad. Sci. U.S.* **68**, 2032 (1971).
8. W. J. Moore and B. Mintz, *Develop. Biol.*, in press.
9. B. Mintz, *J. Anim. Sci.* **27** (Suppl. 1), 51 (1968); B. Mintz, D. A. Hungerford, J. Morrow, in preparation.
10. B. Mintz, *Birth Defects Orig. Art. Ser.* **5**, 11 (1969).
11. E. T. Mystkowska and A. K. Tarkowski, *J. Embryol. Exp. Morphol.* **20**, 33 (1968).
12. E. Witschi, *Arch. Anat. Microscop. Morphol.* **54**, 601 (1965).
13. A. Raynaud, *ibid.* **39**, 518 (1950); D. Price and E. Ortiz, *Organogenesis* (Holt, Rinehart & Winston, New York, 1965), p. 629.
14. H. F. Stich and T. C. Hsu, *Exp. Cell Res.* **20**, 248 (1960).
15. N. S. Henderson, *J. Exp. Zool.* **158**, 263 (1965).
16. ———, *Arch. Biochem. Biophys.* **117**, 28 (1966); W. W. Baker and B. Mintz, *Biochem. Genet.* **2**, 351 (1969).
17. R. D. Platz and H. G. Wolfe, *J. Hered.* **60**, 187 (1969).
18. B. J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
19. M. Domon and B. Mintz, in preparation.
20. C. W. Bardin, J. E. Allison, A. J. Stanley, L. G. Gumbreck, *Endocrinology* **84**, 435 (1969).
21. W. J. Welshons and L. B. Russell, *Proc. Nat. Acad. Sci. U.S.* **45**, 560 (1959).
22. Supported by PHS grants HD-01646, CA-05903, and RR-05539, and by an appropriation from the Commonwealth of Pennsylvania.
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Parasympathetic Ganglia: Activation of an Adrenergic Inhibitory Mechanism by Cholinomimetic Agents

Abstract. *Electrical stimulation of the sympathetic nerves to the urinary bladder or the intraarterial administration of the cholinomimetic substances acetylcholine or methacholine produced adrenergic inhibition in parasympathetic ganglia on the surface of the bladder. The inhibition appeared to be mediated, at least in part, via adrenergic inhibitory neurons located in the pelvic plexus. Atropine blocked the inhibitory response to injected cholinomimetic agents but did not alter the response to stimulation of the sympathetic nerves. Thus, the inhibitory neurons can be activated via both muscarinic and nonmuscarinic receptors, the latter being of primary physiological importance.*

Since the discovery by Marrazzi (*1*) that epinephrine depressed transmission in the superior cervical ganglion, there has been considerable interest in the possible role of the catecholamines as inhibitory transmitters in autonomic ganglia (*2*). The first electrophysiological evidence for such a role was obtained by Eccles and Libet (*3*). These investigators showed that repetitive stimulation of the preganglionic nerves to the curarized superior cervical ganglion of the rabbit produced a hyperpolarizing ganglionic potential (the P-potential) which was blocked by an α-adrenergic blocking agent or by atropine. They suggested that preganglionic fibers to the ganglion excited chromaffin cells, which in turn released an adrenaline-like substance. This substance was believed to act on the ganglion cells to produce the hyperpolarizing response. To account for the blockade of the P-potential by atropine, they proposed that transmission at the synapses on the chromaffin cells was cholinergic and was mediated via muscarinic receptors. The presence of chromaffin-like cells in close apposition to sympathetic ganglion cells (*4*) led to the proposal that the former might function as adrenergic inhibitory interneurons (*4*, *5*).

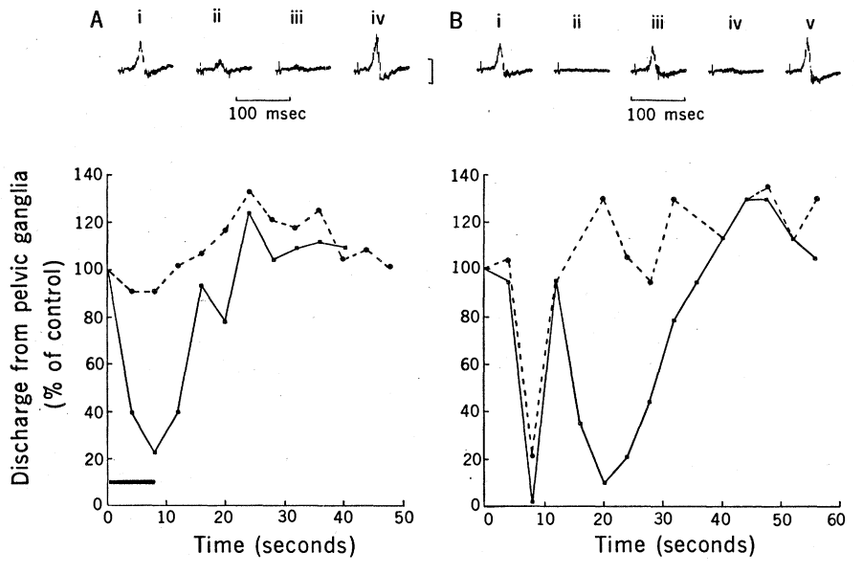

Fig. 1. Time course of the inhibitory effects in pelvic ganglia of (A) electrical stimulation (20 volts, 20 cycle/sec) of the hypogastric nerve (HGN-Stim) and of (B) injected methacholine. (A) Record i is the control discharge recorded on a vesical postganglionic nerve filament in response to submaximal stimulation of the pelvic nerve (2 volts, 0.5 cycle/sec). Records ii, iii, and iv are taken 4 seconds after HGN-Stim on, 2 seconds after HGN-Stim off, and 18 seconds after HGN-Stim off, respectively. Vertical calibration is 200 μv. Below is a graph of the depression by HGN-Stim (20 volts, 20 cycle/sec) of the discharge from pelvic ganglia. The bar indicates HGN-Stim. Dihydroergotamine (200 μg) was administered; the solid line shows data obtained before this drug was given, and the dashed line shows data obtained after it was given. (B) Record i is the control discharge, and records ii, iii, iv, and v are taken 8, 12, 20, and 44 seconds, respectively, after an injection of methacholine (5 μg). Vertical calibration is 200 μv. Below is a graph of the depression by methacholine (5 μg) of the discharge from pelvic ganglia. Dihydroergotamine (200 μg) was given; solid and dashed lines are as explained for A.

Evidence has also been obtained for an adrenergic inhibitory mechanism in parasympathetic ganglia. Histofluorescence studies (*6*) revealed that parasympathetic ganglion cells in the bladder wall received synaptic contacts from adrenergic neurons, located in the pelvic plexus. Stimulation of the sympathetic nerves (hypogastric) to the urinary bladder inhibited transmission in vesical parasympathetic ganglia and this inhibition was mediated via α-adrenergic receptors (*7*). Since the intensity of stimulation necessary to elicit the inhibition was below the threshold for activating unmyelinated postganglionic fibers in the hypogastric nerves, it was suggested that the inhibitory pathway was composed of preganglionic axons that passed directly through the paravertebral and prevertebral sympathetic ganglia to make synaptic connections with adrenergic inhibitory neurons in the pelvic plexus. The proposed inhibitory pathway is shown in Fig. 2B. In the present experiments we have obtained further evidence for the existence of this pathway.

Experiments were performed on 32 cats anesthetized with a mixture of sodium diallylbarbiturate (70 mg/kg), urethan (280 mg/kg), and monoethylurea (280 mg/kg), administered intraperitoneally. The parasympathetic (pelvic) and sympathetic (hypogastric) innervations (*8*) to the urinary bladder were exposed through a midline abdominal incision and dissected free from underlying connective tissue. Preganglionic nerves to the inferior mesenteric ganglia (IMG), the hypogastric nerves, and the pelvic nerves were mounted on bipolar electrodes for stimulation. Ganglia on the surface of the bladder were identified, and postganglionic fibers were positioned on electrodes for monophasic recording. The central parasympathetic outflow to the bladder was blocked by transection of the spinal cord at L-1 (*9*). The inferior mesenteric artery was cannulated for close intraarterial administration of drugs, and the external iliac arteries were ligated to increase the amount of drug reaching the ganglia.

Electrical stimulation of preganglionic fibers in the pelvic nerves produced a postganglionic discharge, the amplitude of which was used as a monitor of ganglionic transmission. Repetitive stimulation (5 to 20 cycle/sec) of either the hypogastric nerve or preganglionic nerves to the IMG inhibited transmission in the ipsilateral pelvic ganglia (Fig. 1A). To demonstrate the existence of inhibitory fibers passing directly through the IMG, nicotine was applied to the surface of the ganglia to block ganglionic transmission (*10*). Topical

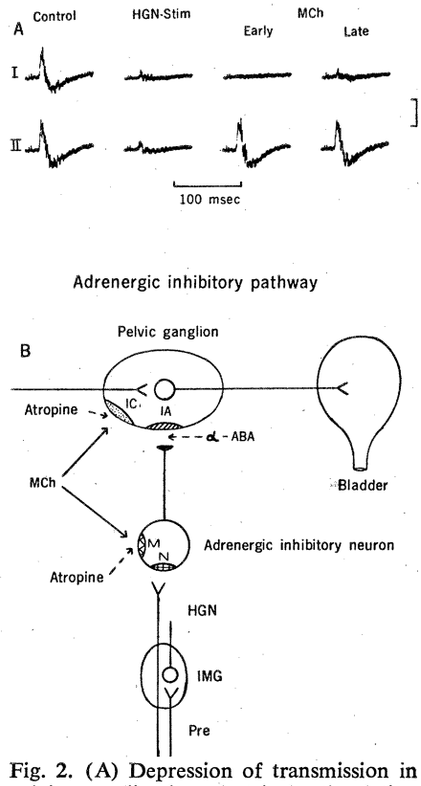

Fig. 2. (A) Depression of transmission in pelvic ganglia by electrical stimulation (5 volts, 20 cycle/sec) of the hypogastric nerve (*HGN-Stim*), and by injected methacholine (*MCh*) (5 μg). *Early* and *Late* refer to the two phases of methacholine depression. Recordings were made on a postganglionic nerve filament on the surface of the urinary bladder before (I) and after (II) the intraarterial administration of atropine (10 μg). Discharges were elicited by submaximal stimulation of the ipsilateral pelvic nerve (3 volts, 0.5 cycle/sec). Vertical calibration is 200 μv. (B) Diagram of the proposed adrenergic inhibitory pathway, which is activated by electrical stimulation of the sympathetic nerves (hypogastric, *HGN*; and preganglionic, *Pre*) or by an administered cholinomimetic agent (methacholine, *MCh*). Muscarinic (*M*) and nonmuscarinic (*N*) receptors are depicted on adrenergic inhibitory neurons, and inhibitory cholinoceptive (*IC*) and inhibitory adrenoceptive (*IA*) sites are shown in the pelvic ganglion. The latter receptors are not shown on any particular neural element since their specific location has not been determined. The α-adrenergic blocking agent is shown as *α-ABA*, and the inferior mesenteric ganglion is shown as *IMG*. The preganglionic nerve to the IMG is shown passing directly through the ganglion to synapse with adrenergic inhibitory neuron.

application of nicotine (0.1 percent solution) completely blocked IMG transmission in two experiments but did not block the inhibition elicited by stimulation of hypogastric or preganglionic nerves. In two other experiments, however, similar applications of nicotine selectively reduced the inhibition caused by preganglionic stimulation but did not affect that caused by hypogastric stimulation. These results indicate that part of the preganglionic input to the IMG passes directly through the ganglion to make synaptic contacts with more peripherally located adrenergic inhibitory neurons (Fig. 2B).

It is generally accepted that acetylcholine is the transmitter released by all sympathetic preganglionic fibers and that transmission in autonomic ganglia is mediated primarily via nicotinic receptors. It was of interest, therefore, to determine whether transmission at synapses on the adrenergic inhibitory neurons was also mediated via a cholinergic mechanism. We attempted to activate the adrenergic inhibitory neurons by administering ganglionic stimulating agents. Close intraarterial injections of nicotine (10 to 20 μg) and tetramethylammonium (10 to 20 μg) produced only a facilitation of pelvic ganglionic transmission (50 to 150 percent increase in spike amplitude). On the other hand, acetylcholine (2.5 to 20 μg) and methacholine (2.5 to 20 μg), a muscarinic stimulant, produced a biphasic depression of ganglionic transmission (Fig. 1B). This was followed in most experiments by a facilitation lasting 1 to 4 minutes. Like the adrenergic inhibition produced by hypogastric nerve stimulation, the late depression elicited by acetylcholine and methacholine was completely antagonized by the α-adrenergic blocking agent dihydroergotamine (100 to 250 μg), whereas the early depression was unaffected (Fig. 1B). After administration of dihydroergotamine the facilitation produced by these cholinomimetic agents occurred earlier and was often enhanced. In contrast to the selective action of dihydroergotamine, atropine (1 to 4 μg) blocked both phases of the inhibition (Fig. 2A) and the late facilitation. However, atropine (0.1 to 0.5 mg) in doses sufficient to antagonize the biphasic depression caused by methacholine had no effect on the ganglionic inhibition produced by hypogastric stimulation (Fig. 2A).

The biphasic depression produced

by acetylcholine and methacholine was unaffected by transection of the hypogastric nerves peripheral to the IMG or by the occlusion of the renal arteries and veins. These results suggest that the ganglionic depression by acetylcholine and methacholine was not due to activation of neurons in the IMG or to release of catecholamines from the adrenal medulla but to the activation of adrenergic inhibitory neurons in the pelvic plexus.

Thus, these experiments provide further evidence for the existence of an adrenergic inhibitory mechanism in vesical parasympathetic ganglia (*7*). We have shown that intraarterial administration of the cholinomimetic agents acetylcholine and methacholine can activate such a mechanism. These substances produced a biphasic depression of ganglionic transmission: an early depression, which was presumably a direct effect of the agents on ganglionic transmission, and a late depression, which was dependent upon the release of endogenous catecholamines. The late inhibition was blocked by both an α-adrenergic blocking agent and by atropine. We conclude therefore that the cholinomimetic substances activated the adrenergic inhibitory neurons via muscarinic receptors (Fig. 2B).

On the other hand, atropine did not block the ganglionic inhibition evoked by electrical stimulation of sympathetic pregaglionic fibers. This result indicates that the adrenergic inhibitory neurons are synaptically excited via nonmuscarinic receptors (Fig. 2B). By analogy with synaptic transmission in other peripheral ganglia, it seems likely that the latter receptors are of the nicotinic type. However, the injection of nicotinic stimulants did not produce adrenergic inhibition. Nevertheless, these substances might have activated the adrenergic inhibitory neurons, but the inhibition was undetectable because of the predominant excitatory action of nicotinic stimulants directly on the parasympathetic ganglia.

The direct effects of the cholinomimetic agents on transmission in bladder ganglia underscore the complexity of drug actions at ganglionic synapses (*2*). Acetylcholine and methacholine produced a direct (early) depression of transmission and then a late facilitation, both of which were blocked by atropine. Similar effects have been observed (*11*) in sympathetic ganglia after the administration of these agents, and there has been considerable speculation (*2, 3, 5*) about the physiological significance of these effects. The present observations raise the possibility that atropine-sensitive synaptic mechanisms may also be present in parasympathetic ganglia.

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References and Notes

1. A. S. Marrazzi, *J. Pharmacol. Exp. Ther.* **65**, 395 (1939).
2. R. L. Volle, *Pharmacol. Rev.* **18**, 395 (1966); W. C. de Groat, *Circ. Res.* **20-21** (Suppl. 3), 135 (1967).
3. R. M. Eccles and B. Libet, *J. Physiol.* **157**, 484 (1961).
4. K.-A. Norberg and F. Sjöqvist, *Pharmacol. Rev.* **18**, 743 (1966); M. A. Grillo, *ibid.*, p. 387; T. H. W. Williams, *Nature* **214**, 309 (1967); M. R. Matthews and G. Raisman, *J. Anat.* **105**, 255 (1969); D. Jacobowitz, *Fed. Proc.* **29**, 1929 (1970).
5. B. Libet, *Fed. Proc.* **29**, 1945 (1970).
6. B. Hamberger and K.-A. Norberg, *Int. J. Neuropharmacol.* **4**, 41 (1965).
7. W. C. de Groat and W. R. Saum, *Fed. Proc.* **30**, 656 (1971); *Nature New Biol.* **231**, 188 (1971).
8. The parasympathetic pathway represents the principal excitatory input to the bladder, and the sympathetic pathway is thought to be primarily inhibitory [M. Kuru, *Physiol Rev.* **45**, 425 (1965)].
9. W. C. de Groat and R. W. Ryall, *J. Physiol.* **200**, 87 (1969).
10. J. N. Langley, *ibid.* **19**, 131 (1895).
11. C. Takeshige, A. J. Pappano, W. C. de Groat, R. L. Volle, *J. Pharmacol. Exp. Ther.* **141**, 333 (1963).
12. Supported by NIH grant NB 07923. We thank Miss E. La Rocco for expert technical assistance.

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Neuronal Correlates of Eye Movements in the Visual Cortex of the Cat

Abstract. *About 10 percent of the cells in the visual cortex of awake cats do not respond to stationary parallel stripes in any orientation or to stripes moving across the visual field in any direction at a moderate speed (up to 132 degrees per second), but these cells are either excited or inhibited during saccadic eye movements when the animal faces a patterned visual environment. Of nineteen such cells tested in total darkness, seven discharged in association with eye movements. For saccade-related discharges, the latency during retinal stimulation is typically shorter than the latency in total darkness.*

Psychologists and neurophysiologists alike are intrigued by the ability of the brain to distinguish between image motion on the retina brought about by object movement and by active movement of the eye. The most promising hypothesis for a mechanism which might allow the brain to differentiate between the two types of image motion was that of a corollary discharge (*1*) that somehow modifies the visual input during an eye movement. In studies of single units, neither in the visual cortex (*2*) nor in the frontal eye fields (*3*) have neurons been found whose discharge pattern before or during eye movements could be interpreted as a corollary discharge. Only in the superior colliculi (*4*) and in the lateral geniculate body (*5*) are neuronal responses typically related to eye movements. In area 17 of awake monkeys, some neurons in the visual cortex are excited or inhibited during rapid eye movements as well as during fast object movements (angular velocity as great as 900° sec^{-1}). But these neurons did not discharge in relation to eye movements during total darkness (*2*).

More than 300 neurons in the visual cortex of awake cats were studied and classified into four types according to their dominant response during image movements (*6*). These types were (i) units that responded continuously to stationary gratings (parallel stripes) of a certain orientation (24 percent); (ii) units that responded to stripes of an optimal orientation that were moving at right angles to that orientation at a moderate speed, 12° to 100° sec^{-1} (25 percent); (iii) units that could be driven only by undefinable movements, such as shadows of wiggling fingers on the uniformly illuminated projection screen (20 percent); and (iv) units that only showed a response during a saccadic eye movement while the cat was looking at a large contrast pattern, such as a checkerboard (10 percent). Some of the type iv neurons also fired in association with rapid eye movements during complete darkness. The remaining 21 percent of neurons were unclassifiable.

A total of 357 cortical neurons were recorded in 90 experiments on 20 unanesthetized, awake cats. The surgery