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Inhibition by Colchicine and by Vinblastine of Acetylcholine-Induced Catecholamine Release from the Adrenal Gland: an Anticholinergic Action, Not an Effect upon Microtubules

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SUMMARY

We have confirmed that colchicine (1 mM) or vinblastine (100 μM) inhibits the acetylcholine-induced release of catecholamines from perfused bovine adrenal glands. This is an anticholinergic effect of these drugs and cannot be interpreted as evidence that microtubules participate in the release of amines, because neither colchicine nor vinblastine reduced catecholamine released by a high concentration of K^+ or by angiotensin. Furthermore, both colchicine (1 mM) and vinblastine (100 μM) blocked transmission through the superior cervical ganglion of the cat by a postsynaptic anti-acetylcholine action.

Poisner and Bernstein (1) recently showed that colchicine and certain *Vinca* alkaloids inhibit the release of catecholamines from perfused adrenal glands that are stimulated by acetylcholine or by nicotine. These authors interpreted their finding as evidence that microtubules are involved in the process of secretion of amines, because colchicine, vinblastine, and vincristine have been shown to affect microtubules (2–4). The present experiments confirm that colchicine and vinblastine reduce the acetylcholine-induced release of catecholamines from the adrenal gland, but suggest that this effect is the result of an anticholinergic action of these drugs, rather than the result of a generalized effect upon microtubules.

Bovine adrenal glands were obtained from an abattoir and were perfused as described before (5); the release of catecholamines was stimulated by switching the perfusion to a medium containing acetylcholine (100 μM), a high concentration of K^+ (56 mM; in these experiments the concentration of NaCl was reduced to maintain isotonicity), or angiotensin (100 μM). The amount of catecholamine in the perfusate was assayed by the trihydroxyindole fluorometric method (6). Cat superior cervical ganglia were perfused by Kibjakow's (7) method as described previously (8). Acetylcholine release was measured by bioassay on the blood pressure of the eviscerated cat. In these experiments perfusion was carried out with Krebs solution containing eserine (10 μM) as the anticholinesterase, and choline (10 μM) to maintain acetylcholine synthesis. In other experiments the ganglion-stimulatory effect of acetylcholine was tested by injecting the drug directly into the arterial cannula, and in these experiments perfusion was performed with choline (10 μM) in Krebs solution. The cut preganglionic sympathetic nerve was stimulated supramaximally (3–8 V, 0.3 msec, 5 Hz). Isometric contractions of the ipsilateral nictitating membrane were recorded to assess ganglionic transmission. Colchicine was purchased from Fluka (Swit-

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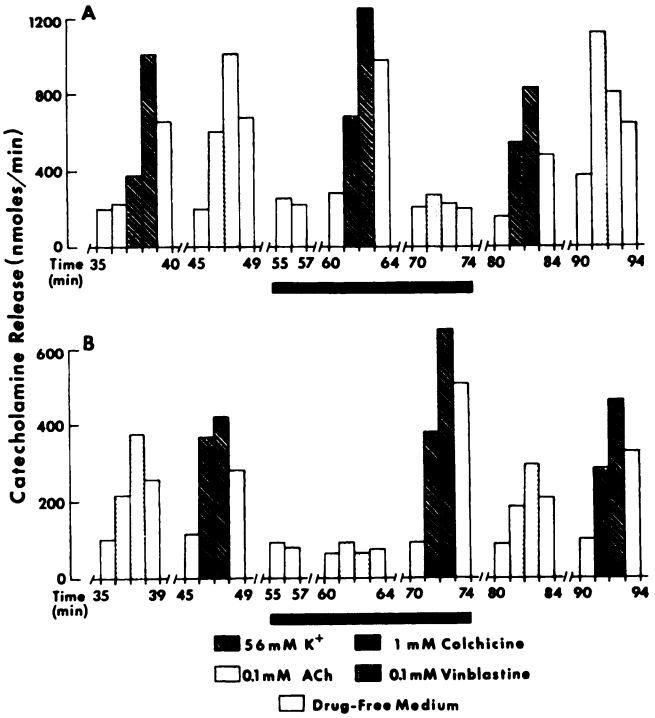

FIG. 1. *Effect of acetylcholine (ACh) and potassium on catecholamine output from bovine adrenal glands perfused in the presence and absence of colchicine or vinblastine*

Adrenal glands were perfused (15 ml/min) at room temperature (25°). The perfusate was collected at 1-min intervals, and catecholamines were assayed as indicated in the text. The perfusion medium contained colchicine (A) or vinblastine sulfate (B) during the periods indicated by the horizontal bars.

zerland), and vinblastine sulfate was generously provided by Dr. M. Ferron (Eli Lilly and Company, Canada).

The demonstration by Poisner and Bernstein (1) that colchicine reduces the acetylcholine-induced release of catecholamines from perfused adrenal glands was readily confirmed; however, colchicine did not inhibit the K^+-induced release of amines. The results of a typical experiment showing these effects of colchicine are illustrated in Fig. 1A. It is clear that colchicine (1mM) almost completely prevented the acetylcholine-evoked release of amines, but enhanced rather than reduced the release by K^+. In six experiments, colchicine (1 mM) reduced the acetylcholine-induced catecholamine release by 65–100%; lower concentrations of drug were less effective. Colchicine at 100 μM inhibited release by 35% (one experiment), and at 10 μM did not affect release (one experiment). Three experiments showed that colchicine (1 mM) did not reduce the amount of catecholamines released by a high concentration of K^+; in two of these experiments K^+-induced release was potentiated (70–75%), and in the other experiment it was not. One other experiment showed that colchicine (1 mM) did not reduce catecholamine released by angiotensin (100 μM).

Similar results were obtained in experiments which used vinblastine (100 μM) instead of colchicine. Figure 1B shows that vinblastine abolished acetylcholine-induced amine release but increased K^+-induced amine release; this was confirmed by two other experiments.

A logical interpretation of the inhibition by colchicine and vinblastine of the acetylcholine-induced release of amines but not of K^+-induced release is that these drugs, in the concentrations used, have anticholinergic activity. This was tested directly using the superior cervical ganglion of the cat: auto-

nomic ganglia and the adrenal medulla are embryologically and pharmacologically similar. Three experiments clearly showed that colchicine blocked ganglionic transmission; a reduced response of the nictitating membrane to preganglionic nerve stimulation was apparent 2–3 min after perfusion was switched to a solution containing colchicine (1 mM), ganglion blockade was 60–80% complete after 10 min of exposure to the drug, and recovery was complete 8–10 min after switching to perfusion with drug-free medium. The experiment illustrated in Fig. 2 demonstrates that ganglion blockade by colchicine was not the result of reduced transmitter output; acetylcholine released by preganglionic nerve stimulation (5 Hz for 3 min) was normal when colchicine had clearly impaired ganglionic transmission. Spoor and Ferguson (9) previously suggested that colchicine (1 mM) blocks neuromuscular transmission in the frog by a tubocurarine-like action.

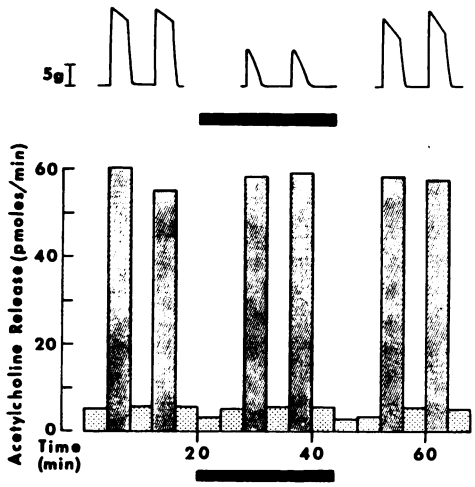

FIG. 2. *Lack of effect of a ganglion-blocking concentration of colchicine upon acetylcholine release from cat superior cervical ganglion*

Acetylcholine output (lower) and contractions of the nictitating membrane (upper) during preganglionic nerve stimulation (5 Hz for first 3 min of a 4-min collection period) are shown. Acetylcholine output at rest is indicated by the stippled columns; output during stimulation is indicated by the hatched columns. Ganglia were perfused with Krebs solution containing eserine (10 μM) and choline (10 μM); perfusion was switched to the same medium containing colchicine (1 mM) during the periods indicated by the horizontal bars.

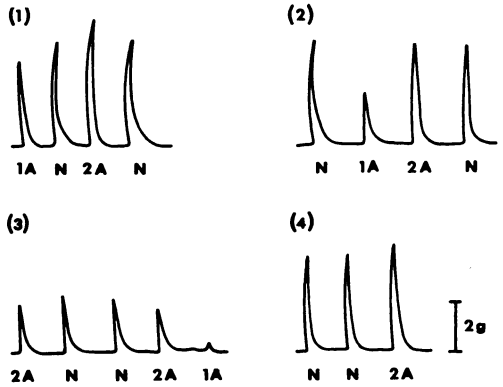

FIG. 3. *Ganglion-blocking action of vinblastine*

Cat superior cervical ganglion was perfused with Krebs solution containing choline (10 μM), and contractions of the nictitating membrane were measured. Responses were elicited by injecting acetylcholine close to the ganglion (*1A* = 1 μg; *2A* = 2 μg) or by preganglionic nerve stimulation (*N* = 5 Hz for 5 sec). *1*, perfusion with drug-free medium; *2*, 10 min after switching to a medium containing vinblastine sulfate (50 μM); *3*, 10 min after switching to a medium containing vinblastine sulfate (100 μM); *4*, 15 min after switching back to the normal medium.

The ganglion-blocking action of vinblastine is illustrated in Fig. 3. In this experiment the response of the nictitating membrane to preganglionic nerve stimulation or to acetylcholine injected close to the ganglion was measured. It is clear that vinblastine (100 μM) depressed ganglionic transmission by a postsynaptic action, because the effects of nerve stimulation and of injected acetylcholine were both depressed. Vinblastine was somewhat more effective in reducing the response to exogenous acetylcholine, for vinblastine (50 μM) reduced the response to injected acetylcholine without affecting the response to nerve stimulation. Two other experiments confirmed these results.

The present experiments demonstrate that the effect of colchicine and vinblastine on catecholamine release from the adrenal glands during acetylcholine stimulation can be explained by anticholinergic actions of these two drugs. The demonstration that D_2O potentiates the release of catecholamines by nicotine (1) remains unexplained; the stabilization of microtubules by D_2O has been shown to inhibit glucose-induced release

of insulin from the pancreas (10), and D_2O inhibits the release of norepinephrine from granules isolated from splenic nerves (11). The role of microtubules in hormone release is still unclear; prolonged exposure to colchicine reduces the K^+-induced release of ACTH from adenohypophyses, but ACTH released by an extract of hypothalamus-stalk-median eminence is not blocked by colchicine (12).

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