Short communications

Colchicine inhibits adrenal medullary secretion evoked by acetylcholine without affecting that evoked by potassium

W. W. DOUGLAS and M. SORIMACHI

Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, U.S.A.

In perfused rabbit adrenal glands, colchicine (500 μM) inhibited the catecholamine secretion evoked by acetylcholine (20 μg/ml) but not that evoked by excess potassium (60 mM). Since both stimuli are believed to release catecholamines ultimately by the same secretory process, exocytosis, it is concluded that these inhibitory effects of colchicine exerted against acetylcholine result from an action early in the process of stimulus-secretion coupling, possibly at the level of the acetylcholine receptor, and that it is inappropriate to use such inhibitory effects to support the view that secretion proper (exocytosis) in chromaffin cells involves colchicine-sensitive elements such as microtubules.

Lacy, Howell, Young & Fink (1968) suggested that microtubules, or like elements, may be involved in secretion of insulin by emiocytosis (exocytosis) and supported their concept with evidence that colchicine, a drug whose disrupting actions on microtubules is well documented (Borisy & Taylor, 1967), depressed the output of insulin from pancreatic β-cells stimulated, *in vitro*, with glucose (see also Malaisse-Lagae, Greider, Malaisse & Lacy, 1971). Experiments on other secretory cells, stimulated by various means, have not always revealed such sensitivity to colchicine (Kraicer & Milligan, 1971; Schofield & Cole, 1971), but where significant inhibition has been observed this has generally been considered as support for the involvement of microtubules or related elements (Gillespie, Levine & Malawista, 1968; Kraicer & Milligan, 1971; Levy & Carlton, 1971; Schofield & Cole, 1971). The experiments of Poisner & Bernstein (1971) fall into this latter category. They found that colchicine (500 μM) caused a rapid and reversible inhibition (by about 50%) of catecholamine secretion from perfused ox adrenal glands stimulated with acetylcholine or nicotine. More recently, we observed that cytochalasin B, a relatively new drug, many of whose pharmacological effects have been attributed to actions on microfilaments (Wessels, Spooner, Ash, Bradley, Luduena, Taylor, Wrenn & Yamada, 1971), is even more effective in inhibiting adrenal medullary responses to acetylcholine. However, our analysis revealed that responses to excess potassium were not so strongly inhibited. This suggested that cytochalasin might owe some of its inhibitory effect against acetylcholine to actions at the receptor level and raised the question whether the same might be true of colchicine. Our results, described briefly in a recent communication to the Society (Douglas & Sorimachi, 1972) have shown that colchicine has an even more striking differential effect, inhibiting adrenal medullary responses to acetylcholine strongly but having little or no influence on responses to potassium.

Methods.—Male New Zealand rabbits weighing about 2·5 kg were anaesthetized with sodium pentobarbital (35 mg/kg, i.v.). The left adrenal gland, acutely denervated, was perfused by the aortic route and the effluent collected from the adrenal vein following the general procedure adopted for cat glands by Douglas & Rubin (1961) as modified by Sorimachi (1968). Catecholamines were assayed by the fluorometric method according to Anton & Sayre (1962). The standard perfusion medium, equilibrated with 0_2 at room temperature (23° C), contained (mM): NaCl, 150; KCl, 6; $CaCl_2$, 2; Na_2HPO_4-NaH_2PO_4 buffer (pH 7·0), 3; glucose, 11. In high K medium, [K] was raised to 60 mM and [Na] lowered by a corresponding amount to 96 mM (Douglas & Rubin, 1961). Secretory responses to such high K medium seem to be largely due to the direct depolarizing action of K (Douglas, Kanno & Sampson, 1967a) but to minimize possible indirect effects of K resulting from release of acetylcholine from secretomotor terminals in the gland the high K medium contained hexamethonium (10 μg/ml) and atropine (1 μg /ml) to block both nicotinic and muscarinic receptors (Douglas & Rubin, 1961; Douglas & Poisner, 1965).

Results.—Fifteen minutes after the perfusion was begun the glands were stimulated by switching from the standard perfusion medium to one containing acetylcholine (20 μg/ml) or excess K (60 mM) for 1 minute. The catecholamine (adrenaline plus noradrenaline) discharge during this period and the following 2 min was taken as a measure of the secretory response. Three successive responses to one type of stimulus, acetylcholine or K, were obtained at 15 min intervals and the sequence was then repeated using the other stimulus, K or acetylcholine respectively, on the same gland. During the second stimulation period of each set of three, and for the preceding 9 min, colchicine (500 μM) was present in the medium. Two typical triads of responses are illustrated in Fig. 1 which depicts our main finding that colchicine significantly reduced responses to acetylcholine but not those to K. In four experiments of this sort, secretory responses to acetylcholine in the presence of colchicine ranged from 30 to 71% of the mean of the preceding and following control responses (46±9%, mean±S.E.). By contrast, colchicine in the same concentration and tested in the same way on the same glands had little or no effect on catecholamine secretion to K, responses in the presence of colchicine ranging from 89 to 100% of the mean of the controls (95±3%, mean±S.E.).

Discussion.—In the present study we have used rabbit adrenal glands perfused through their arteries whereas Poisner & Bernstein (1971) used bovine adrenals perfused backwards through the vein, but in other respects our experiments are very similar to theirs: the concentration of colchicine used (500 μM) is the same, as is the method of testing the drug (save that we exposed the glands to colchicine for 9 min before stimulation rather than 4 min). Our results indicating that colchicine in this concentration inhibits, reversibly,

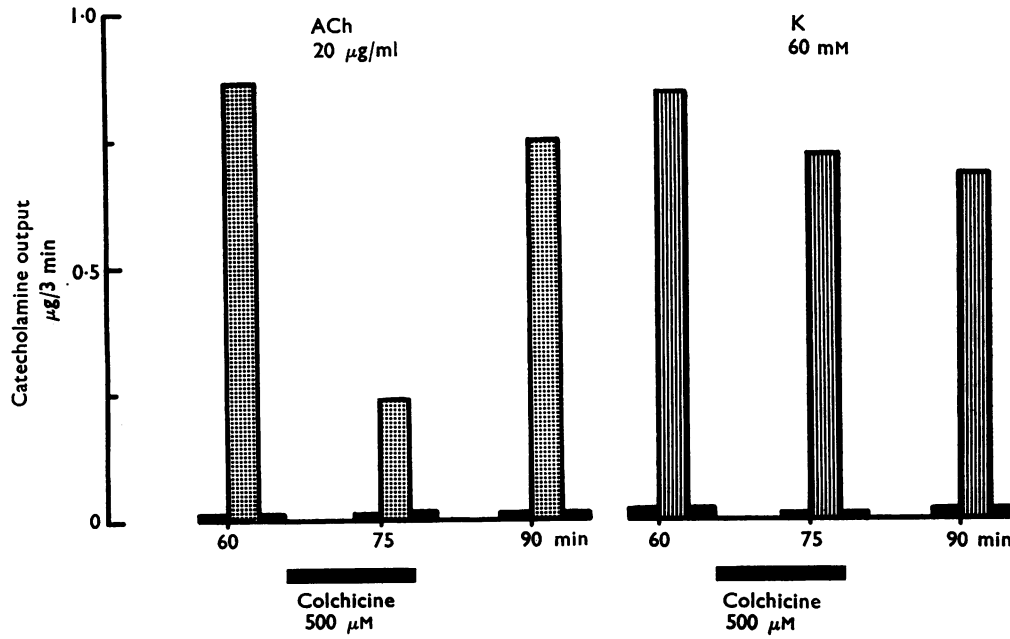

FIG. 1. Representative experiments on perfused rabbit adrenal glands illustrating that colchicine (500 μM) present during the period indicated by the black horizontal bar, inhibits catecholamine output in response to ACh, 20 μg/ml (stippled columns) but has little effect on responses to 60 mM K (striped columns). Stimuli were applied during the first min of the three min periods. The secretion of catecholamines preceding and following each stimulation period is indicated by the black columns. Abscissae: total catecholamine output (adrenaline plus noradrenaline) in the adrenal effluent (μg/3 min). Ordinates: time in min from beginning of perfusion. The two sets of responses were obtained from different glands at similar times after beginning perfusion.

responses to acetylcholine by about 50% agree with theirs obtained with acetylcholine or nicotine. The new finding is that responses to potassium, which they did not test, were inhibited little or not at all by colchicine. The persistence of the response to K suggests to us that colchicine does not act, at least in this concentration, on the secretory process proper (catecholamine extrusion) as Poisner & Bernstein (1971) supposed but at some earlier stage in stimulus-secretion coupling. There is no reason to suspect that hormones are extruded by different mechanisms with the two stimuli: both stimuli depolarize the chromaffin cells (Douglas, Kanno & Sampson, 1967a, b), and appear to act by promoting calcium entry (Douglas & Rubin, 1961, 1963; Douglas & Poisner, 1962; Douglas, Kanno & Sampson, 1967a). Moreover, this calcium-dependent process in each instance seems to be exocytosis (Douglas, 1968). Thus adenine nucleotides, present in chromaffin granules, escape *pari passu* with the catecholamines in response to K as well as to acetylcholine (Douglas, Poisner & Rubin, 1965), and excess K increases the incidence of exocytotic figures in electronmicrographs of adrenal chromaffin cells (Douglas & Nagasawa, 1971 and unpublished results). It seems, therefore, that an explanation of the inhibitory effect of colchicine must be sought in some link in stimulus-secretion coupling prior to hormone extrusion and peculiar to acetylcholine. The most obvious of these concerns the acetylcholine receptor. Certainly the differential blocking effect that involves acetylcholine but not K resembles that produced by an acetylcholine antagonist such as hexamethonium. Furthermore, Spoor & Ferguson (1965) have reported colchicine has curare-like actions at the frog neuromuscular junction. But whatever the precise action of colchicine may prove to be, it seems evident from our analysis that the consequent inhibitory effects restricted to acetylcholine (or acetylcholine-like drugs) and sparing responses to K offer little if any support for the hypothesis that colchicine-sensitive elements, such as microtubules, are involved in the hormone extrusion process proper—exocytosis. Colchicine appears to be yet another pharmacological agent whose usefulness as an analytical tool is compromised by its complexity of action.

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