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The Need for Calcium in Adrenomedullary Secretion Evoked by Biogenic Amines, Polypeptides, and Muscarinic Agents. (31402)

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The stimulant effect of acetylcholine and various nicotinic agents on adrenal medullary secretion is critically dependent on the presence of calcium in the extracellular environment, and it has been suggested that these secretogogues act by promoting an influx of calcium ions into the medullary chromaffin cells(1,2). This hypothesis has been supported by the demonstration of increased calcium^{45} uptake in the medulla exposed to acetylcholine(3), and by studies of the effect of various alkaline metals on medullary secretion(4,5,6).

Since acetylcholine and nicotine-like drugs have many pharmacological properties in common, it is not unexpected that their actions on the adrenal medulla should share a requirement for calcium. However many other substances capable of evoking adrenomedullary secretion have widely different chemical structures and pharmacological properties: these include the polypeptides, bradykinin and angiotensin(7,8,9), the biogenic amines, histamine(10,11,12) and 5-hydroxytryptamine(13), and the muscarinic agents, muscarine, pilocarpine and methacholine(14,15). In the present experiments we have found that each of the secretogogues requires calcium for its stimulant effect on adrenal chromaffin cells. A preliminary account of some of the results has been published(16).

Methods. The experiments were carried out on cats' adrenal glands, acutely denervated and perfused *in situ* through their arteries with Locke's solution or Ca-free Locke's solution by the method previously described (1). In preparing the Ca-free perfusion medium the normal content of $CaCl_2$ (2.2 mM) was omitted; no precautions were taken to remove the traces of calcium present in the other reagents and no chelating agent was added. In each experiment an adrenal gland was perfused first with Ca-free Locke's solution for about 20 minutes and the drug to be tested was introduced for the last 10 to 90 seconds of this period. Then perfusion was switched to Locke's solution and after a further 20 minutes the drug was retested. The perfusates escaping from the adrenal vein during perfusion with the drug-containing solutions, and in the corresponding control periods immediately before each test, were collected in chilled vessels, and were acidified and frozen until they were assayed fluorimetrically for catecholamines (adrenaline plus noradrenaline) by the tri-hydroxy indole method(17). Secretory responses were measured as increments in catecholamine out-

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TABLE I. Responses to Medullary Secretogogues in Presence and Absence of Calcium.

Secretogogue	Concentration (g/ml)	Evoked release of catecholamines (μg/min) In Ca-free 'Locke's solution'	Evoked release of catecholamines (μg/min) In Locke's solution
Polypeptides			
Angiotensin	10^{-5}	.20	1.42
	10^{-4}	.41	2.85
	10^{-5}	.04	3.92
Bradykinin	10^{-4}	.13	.78
	10^{-4}	.03	1.18
Biogenic amines			
Histamine	5×10^{-5}	.20	1.41
	2×10^{-4}	.00	1.81
5-hydroxytryptamine	3×10^{-5}	.01	.47
	3×10^{-4}	.03	.92
Muscarinic agents			
Muscarine	10^{-4}	.01	13.5
Pilocarpine	10^{-4}	.02	1.74
	2×10^{-4}	.12	4.64
Methacholine	10^{-4}	.03	.90
	2×10^{-4}	.05	10.4

put over control catecholamine output. The following drugs were used: angiotensin (Hypertensin CIBA), bradykinin (Sandoz: kindly supplied by Dr. R. Bircher), 5-hydroxytryptamine creatine sulfate, d,l-muscarine iodide (kindly supplied by Dr. P. Greengard), pilocarpine nitrate and methacholine chloride. Except for the peptides, concentrations given in the text refer to the salts.

Results and discussion. All the drugs tested caused a brisk outpouring of catecholamines from the adrenal gland when administered during perfusion with Locke's solution containing the conventional amount of calcium (2.2 mM). But when given during perfusion with Ca-free Locke's solution they had little or no stimulant effect (Table I).

Although muscarine, pilocarpine and methacholine resemble the drugs previously tested in belonging to the family of acetylcholine-like substances, they act through receptors distinct from those activated by the nicotinic drugs: their effects on the adrenal glands are resistant to hexamethonium but are blocked by atropine(15). Moreover there is evidence that each of the other drugs tested, angiotensin, bradykinin, histamine and 5-HT, acts through a different receptor(9,12,18,19). Seemingly then there is a common requirement for calcium in stimulation of medullary chromaffin cells through a variety of receptors. One possible explanation is a common requirement for calcium in the interaction of the various drugs with their receptors on the chromaffin cell. This possibility is suggested by studies on smooth muscle that have led to the view that calcium binding sites may be associated with drug receptor areas there and may facilitate receptor reactivity(20). But is seems more likely that in the present experiments calcium is involved at some stage subsequent to drug-receptor interaction, for a similar calcium dependence has been shown for another medullary secretogogue, excess potassium, whose action is probably not on any 'receptor' but simply to depolarize the cell (21) by lowering the ratio $[K]_i$ to $[K]_o$. Perhaps all these medullary secretogogues act by promoting calcium entry into the chromaffin cells as has been argued for acetylcholine, the nicotinic drugs, and excess K(1,2,3,4,22).

Summary. The following substances evoked catecholamine secretion from cats' adrenal glands perfused with Locke's solution: muscarine, pilocarpine, methacholine, histamine, 5-hydroxytryptamine, angiotensin, and bradykinin. This corroborates earlier findings. However, these substances had little or no effect on secretion when calcium was omitted from the perfusion medium. It is suggested that each of these drugs may act by promoting an influx of calcium into the chromaffin cells as previously proposed for acetylcholine and nicotinic drugs.

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A Study of the Functional Capacities of Spleen Cells After Maintenance *in vitro*.* (31403)

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The ability of cells to retain their unique antigenic character while being held in tissue culture is a well documented fact. Mannick *et al*(1), have shown that rabbit spleen cells may be cultured for as long as 6 weeks and still elicit a second set reaction when used as a first set homograft. That is, the histocompatability antigens remained intact for at least this period. Tumor tissue has also been shown to retain its unique antigenic characteristics for very long periods both *in vitro* and when serially transferred in animals (2).

On the other hand, it has also been demonstrated that severe structural(3) and functional changes can occur in organized tissues that are held in culture for even short periods of time. In our laboratory, it also has been shown that the sensitivity of the structural changes observed in cultured tissue are a function of the complexity of the medium(4) and/or additives such as adrenal corticoids (5).

Little information is available concerning the changes in the functional status of lymphoid tissues *in vitro*. Michaelides and Coons(6) have shown that sensitized lymphocytes are capable of secondary production of antibody for a limited time (4 to 8 days) *in vitro* depending upon the type of antigens added to the culture medium. In addition, Toa and Uhr(7) have shown that primary sensitization of lymphocytes is possible in culture indicating that this aspect of cellular function may be retained for some time *in vitro*. The transformation *in vitro* of peripheral blood lymphocytes to large polyblastic cells in response to the presence of allogenic cells or phytohemagglutinin(8) probably also represents the retention of some degree of functional capacity in culture for at least short periods. However, none of the work cited contains any information concerning the overall functional status of the tissues during and after culture. That is, it is impossible to determine if the lymphatic cells are capable of returning to their original *in vivo* environment and function as a unified cellular system capable of protecting the animal against antigenic insult. Otherwise, the cell population must be viewed as a loosely integrated system as is seen in cell culture capable, at best, of very limited fuction. As a means of measuring the effect of the structural

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