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CATECHOLAMINE SECRETION FROM CRUDE AND PURIFIED ADRENAL  
MEDULLARY  
CHROMAFFIN CELLS OF DOG ADRENAL GLANDS.

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Acetylcholine (ACh) interacts with nicotinic and muscarinic cholinergic receptors on adrenal medulla to secrete catecholamine (CA). The mechanism of muscarinic receptor-mediated CA secretion remains unclear because it is extremely difficult to obtain large quantities of purified adrenal medullary chromaffin cells. This paper reports a method for the preparation of purified chromaffin cells from dog adrenal glands which release CA in response to muscarinic receptor activation. In addition, the characteristics of CA secretion from this purified preparation are compared with those from a crude preparation.

1. Mongrel dogs (weighting 8 to 15kg) were killed by exsanguination after being anesthetized with sodium pentobarbitone (30mg/kg) intravenously, and both adrenal glands were dissected free from surrounding tissue. The glands were sliced, and the cortex was removed. A cell suspension was prepared by collagenase (2 to 2.5mg) digestion of the sliced medulla. Purified chromaffin cells were obtained by centrifugation at  $1500\times g$  of this cell suspension through a percoll linear density gradient (density 1.004 to 1.083) for 30 min at  $4^{\circ}\text{C}$ . The yield of purified chromaffin cells was  $3.4\times 10^5$ / dog. Approximately 80% of the cells were chromaffin cells, as judged by staining with neutral red and DBH (dopamine- $\beta$ -hydroxylase) fluorescent antibodies.
2. ACh ( $10^{-7}$  to  $10^{-4}\text{M}$ ) caused dose dependent CA secretion from the purified chromaffin cells. The  $\text{ED}_{50}$  value of ACh was  $0.9\times 10^{-6}\text{M}$ . Pilocarpine ( $10^{-3}\text{M}$ ) and a high concentration of KCl (56mM), but not nicotine ( $10^{-5}\text{M}$ ), were also effective in releasing catecholamine.
3. Crude chromaffin cells isolated by collagenase digestion of whole adrenal slices also secreted CA by ACh ( $\text{ED}_{50}$  value ;  $1.5\times 10^{-6}\text{M}$ ), but the maximal response was significantly lower than that of purified chromaffin cells. ACh-stimulated CA secretion is markedly inhibited by atropine ( $10^{-6}\text{M}$ ) but little affected by hexamethonium ( $5\times 10^{-4}\text{M}$ ).

The results described above indicate that purified adrenal medullary chromaffin cells of the dog are useful for the study of the mechanism of muscarinic receptor-mediated CA secretion.