Caffeine-Induced Catecholamine Secretion: Similarity to Caffeine-Induced Muscle Contraction[1] (36967)

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Studies on the adrenal medulla and other tissues have demonstrated that the regulation of secretion parallels control of muscle contraction (1, 2). In fact, it has been suggested that the molecular basis for secretion is identical to that of contraction and involves a contractile event (3, 4). Support for this concept comes from studies showing an actomyosin-like protein in the adrenal medulla (2, 4). Caffeine, a methyl xanthine alkaloid, has been extremely valuable as a tool for studying muscle physiology and has helped form present concepts on the regulation of muscle contraction (5). The present report shows that caffeine has a direct stimulant effect on adrenal medullary secretion. Furthermore, factors which modify caffeine action on muscle contraction have comparable effects on caffeine-induced catecholamine release.

Methods. Bovine adrenal glands were obtained from a local slaughterhouse and perfused at room temperature as described previously (6) except that 10 m*M* Tris buffer (pH 7.4) was used instead of bicarbonate buffer and the solutions were saturated with 100% O_2. Catecholamines were assayed by an automated fluorometric method (7). To exclude possible indirect effects of caffeine due to release of acetylcholine from nerve endings within the gland, hexamethonium and atropine were added to the perfusion fluid since these agents block the nicotinic and muscarinic effects of acetylcholine on the adrenal medulla (8). In calcium-loading experiments, control glands were stimulated twice with 40 m*M* caffeine in Locke's solution with a 14 min interval between stimulations. The other set of glands was stimulated initially just as the control glands. Then they were perfused for 5 min with Locke's solution containing 30 m*M* $CaCl_2$ followed by 9 min with a Ca-free Locke's solution containing 1.6 m*M* EGTA.[2] The second response to caffeine was expressed as a percentage of the first in both sets of glands and the results with the calcium-loaded glands were then compared to the values obtained with the control glands. Measurement of free calcium in the perfusates during exposure to caffeine in the calcium-free medium revealed a concentration of less than 0.01 m*M*.

Results and Discussion. The secretory rate during the 4 min period prior to exposure to caffeine in 12 glands ranged from 8 to 20 μg/min; during perfusion with caffeine in these glands for 4 min the rate ranged from 43 to 145 μg/min. The mean increase in catecholamine release was 72.7 μg/min, which represents an increase of 561 $\pm$ 65% above the control secretory rate.

Caffeine is one of the few agents which will stimulate muscle contraction in the absence of extracellular calcium and this result (plus other experiments) has led to the concept that caffeine releases intracellular calcium from membrane-bound stores (5). The adrenal medulla also requires calcium for stimulation by potassium chloride, cholinergic agents, and other substances (9, 10). Experiments were done, using each gland as its own control, to test whether caffeine could evoke catecholamine release in the absence of extracellular calcium. A series of glands was stimulated twice with caffeine: the first stimulation was in the presence of the nor-

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[2] EGTA is (ethylenebis(oxyethylenenitrilo)) tetraacetic acid, a chelator with much greater affinity for calcium than magnesium.

mal concentration of calcium (2.2 m*M*); and the second stimulation, 16 min later, was obtained either during normal calcium perfusion or after perfusion with calcium-free Locke's solution containing 1.0 m*M* EGTA. In 12 control glands in which the calcium concentration was 2.2 m*M* during both periods with caffeine, the second stimulation produced an increase in catecholamine release which was 18.5 ± 1.5% of the first one. In six other glands which were perfused with the calcium-free medium for 16 min before the second stimulation, the corresponding value was 13.6 ± 3.5%. Thus, the stimulation by caffeine was as effective in the absence of extracellular calcium as in its presence, suggesting that caffeine may mobilize intracellular calcium in the adrenal medulla just as it does in muscle (11).

The suggestion that caffeine-induced catecholamine release depends on intracellular calcium is supported by experiments showing that loading the cell with calcium potentiates the effect of caffeine. Four glands were stimulated with caffeine in calcium-free medium following a brief exposure to a 30 m*M* calcium solution as described in *Methods*. The effect of the calcium loading was to increase the response to caffeine. During the stimulation following the calcium loading, the glands responded to caffeine at about twice the secretory rate expected from the results with 4 control glands: 186 ± 28% of the expected response. Thus, while extracellular calcium is not required for stimulation, loading of the calcium stores increased the stimulant effect of caffeine.

The effect of caffeine on muscle contraction is potentiated by thiocyanate (12). To test for comparable effects on the adrenal medulla, experiments were performed on adrenal glands perfused with different concentrations of NaSCN replacing NaCl. The effect of caffeine on catecholamine release was potentiated by increasing concentrations of thiocyanate (Fig. 1).

The present experiments indicate that caffeine acts on the adrenal medulla in a fashion analogous to its action on skeletal muscle. This is consistent with the suggestion that the secretory process involves contraction (2–4) and with reports on calcium-binding by intracellular organelles in the adrenal medulla (13). The action of another methyl xanthine, aminophylline, on adrenal medullary secretion also does not require extracellular calcium but is inhibited by dibucaine, a local anesthetic (14, 15). Since local anesthetics also block the effect of caffeine on muscle contraction and calcium mobilization (16), the parallels between the actions of methyl xanthines on the adrenal medulla and muscle are numerous.

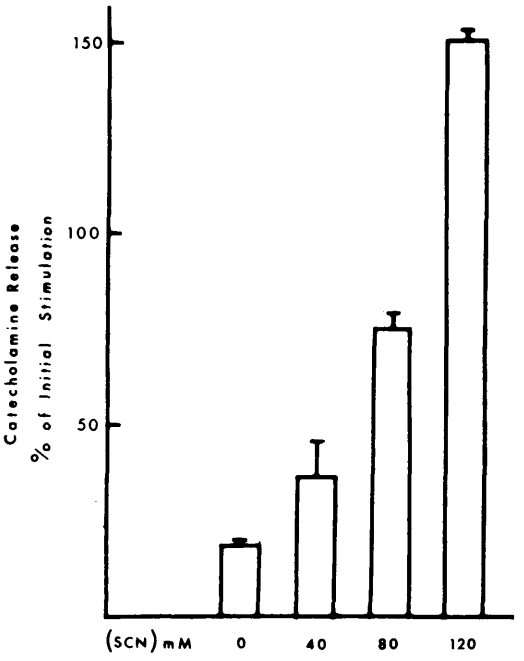

FIG. 1. Effect of thiocyanate on caffeine-induced catecholamine release. Adrenal glands were stimulated twice at 16 min intervals with caffeine (40 m*M*). After the first stimulation, NaCl in the perfusion medium was replaced with varying amounts of NaSCN as indicated. The ordinate represents the caffeine-induced release of catecholamines during the second stimulation period as a percentage of the first. The lines above the vertical bars are the standard errors of the means. For controls, $n = 12$; for SCN values, $n = 3$.

The potency of caffeine in evoking muscle contraction varies among skeletal, smooth, and cardiac muscle. Some muscles respond only weakly even at 50 m*M* caffeine (17). The action of caffeine on the adrenal medulla is not due to osmotic properties since control

experiments indicate that hypertonicity of this magnitude is not a stimulus for catecholamine release.

The role of cyclic AMP in muscle contraction and adrenal medullary secretion is unknown. But since aminophylline is known to increase cyclic AMP levels in the adrenal medulla (18) and is a more potent secretogogue on the adrenal medulla than caffeine (14, 15), studies on the role of cyclic AMP in mobilizing intracellular calcium in secretory tissues are of obvious interest.

Summary. Caffeine directly stimulates catecholamine release from adrenal glands perfused with calcium-free medium. The stimulant effect of caffeine is potentiated by preloading the gland with calcium and also by exposure to thiocyanate. These effects parallel the actions of caffeine on muscle contraction and suggest that catecholamine release can be evoked by mobilization of intracellular calcium.

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