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The Effect of Ca^{++} Omission on the Secretion of Catecholamines and the Incorporation of Orthophosphate-^{32}P into Nucleotides and Phospholipids of Bovine Adrenal Medulla during Acetylcholine Stimulation

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SUMMARY

Bovine adrenal medullary slices were incubated at 30° in Locke's solution containing orthophosphate-^{32}P (50 μCi/ml) with and without Ca^{++}, and were stimulated with acetylcholine (10^{-5} M) in the presence of eserine (10^{-5} M).

The omission of Ca^{++}, as expected, abolished the increment in catecholamine secretion due to acetylcholine stimulation.

Acetylcholine stimulation increased the incorporation of ^{32}P into phospholipids, particularly into phosphatidic acid and into phosphatidylinositol, in both the presence and absence of Ca^{++}. Thus, a lack of correlation between catecholamine release and ^{32}P incorporation into phospholipids upon acetylcholine stimulation was observed.

It has been demonstrated that acetylcholine stimulation increases the incorporation of ^{32}P into the phospholipids of the adrenal medulla (1, 2), particularly phosphatidic acid and phosphatidylinositol. This effect of acetylcholine was due to an increase in the turnover of phosphorus in these two phosphatides, rather than to secondary changes in the specific activity of precursors (2). Since ^{32}P incorporation into phospholipids is also increased in other tissues where acetylcholine seems to be the naturally occurring transmitter (3–6), it was postulated that phospholipids may play an active role in the secretory process (1, 3, 6). The results published in a previous paper (2), showing that following acetylcholine stimulation the ^{32}P

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TABLE 1

Effect of Ca^{++} omission on specific activity of individual phospholipids in the presence and absence of acetylcholine plus eserine

Adrenal medullary slices were incubated with orthophosphate-^{32}P (50 μCi/ml) for 60 min before the addition of acetylcholine (10^{-5} M) plus eserine (10^{-5} M) to the incubation medium, and for 20 min after this addition. Half the slices were incubated in Ca^{++}-free Locke's solution. The radioactivity shown accounted for 95.4 ± 6.5% of the total radioactivity in the lipid extract.

Fraction	Ca^{++}–Locke's solution		Ca^{++}-free Locke's solution	
	Control	Acetylcholine + eserine	Control	Acetylcholine + eserine
	($cpm \times 10^{-2}$)/μmole P		($cpm \times 10^{-2}$)/μmole P	
Lysophosphatidylcholine	12 ± 2.1[a] (7)[b]	13 ± 2.4 (7)	13 ± 2.9 (6)	14 ± 3.2 (7)
Sphingomyelin	22 ± 2.7 (7)	21 ± 2.8 (7)	22 ± 2.5 (6)	24 ± 2.0 (7)
Phosphatidylserine	53 ± 10.0 (7)	49 ± 5.6 (7)	52 ± 8.0 (6)	50 ± 6.4 (7)
Phosphatidylcholine	45 ± 5.8 (7)	65 ± 8.2 (7)	48 ± 5.0 (6)	67 ± 9.8 (7)
Phosphatidylinositol	324 ± 38 (7)	874 ± 51 (7)	316 ± 38 (6)	879 ± 40 (7)
Phosphatidylethanolamine	7 ± 1.3 (7)	8 ± 1.3 (7)	7 ± 2.2 (6)	7 ± 1.0 (7)
Phosphatidic acid	40 ± 9.7 (7)	136 ± 14 (7)	48 ± 7.1 (6)	140 ± 14 (7)
Origin	3138 ± 102 (7)	4832 ± 118 (7)	3332 ± 60 (6)	4792 ± 122 (7)

[a] Mean ± standard error.

[b] The number of experiments is indicated in parentheses.

incorporation into phosphatidic acid and phosphatidylinositol became apparent after catecholamine release reached its maximum, prompted questioning of the relation between this metabolic effect of acetylcholine and the acetylcholine-evoked catecholamine secretion. It was decided then to examine this relationship further by comparing the effects of Ca^{++} on the two acetylcholine-dependent responses of the adrenal medulla.

Adult bovine adrenal glands were used within 40 min after their removal. Medullary slices were prepared and incubated, and their catecholamine, orthophosphate, nucleotide, and lipid contents were assayed as previously described (2).

It is known that Ca^{++} omission abolishes the increase in catecholamine secretion from perfused adrenal glands in response to acetylcholine stimulation (7). Under the present experimental conditions, when the adrenal medullary slices were incubated in the absence of Ca^{++}, acetylcholine (10^{-5} M) stimulation, as expected, did not produce any increase in catecholamine secretion (catecholamine release, in micromoles per 100 mg per minute: Ca^{++}–Locke's, 44.8 ± 1.4; Ca^{++}–Locke's + acetylcholine, 158.7 ± 1.6; Ca^{++}-free Locke's, 44.3 ± 1.4; Ca^{++}-free Locke's + acetylcholine, 43 ± 1.4).

^{32}P was incorporated into the individual phospholipids of the adrenal medulla at the same rate in both Ca^{++}-containing and Ca^{++}-free solutions (Table 1). As in the presence of Ca^{++}, acetylcholine stimulation in Ca^{++}-free Locke's solution produced an increase in the incorporation of ^{32}P mainly into phosphatidic acid (192%) and phosphatidylinositol (178%), and minor incorporation into phosphatidylcholine (40%), with 44% incorporation into the phosphorus-containing spot that remained at the origin of the chromatograms. When net specific activities were determined, it was found that acetylcholine stimulation increased the specific activities of these same phosphatides (see Table 1).

No differences were found in the content of individual phospholipids under any of the experimental conditions.

Experiments were also carried out to see whether the increased labeling found in the phospholipids incubated in Ca^{++}-free medium after acetylcholine stimulation was due to changes in permeability to ^{32}P or to changes in the specific activity of nucleotides as a result of Ca^{++} omission. Table 2

TABLE 2

Effect of Ca^{++} omission on levels and specific activities of tissue orthophosphate and nucleotide phosphorus in the presence and absence of acetylcholine plus eserine

Adrenal medullary slices were incubated with orthophosphate-^{32}P (50 μCi/ml) for 60 min before the addition to the incubation medium of acetylcholine (10^{-5} M) plus eserine (10^{-5} M), and for 20 min after this addition. Half the slices were incubated in a Ca^{++}-free medium.

	Level		Specific activity	
Incubation medium	Orthophosphate	Nucleotide phosphorus	Tissue orthophosphate	Tissue nucleotide phosphorus
	μmoles P/g fresh medulla		*(cpm × 10^{-4})/μmole P*	
Ca^{++}–Locke's solution with acetylcholine + eserine	15.90 ± 1.45[a] (12)[b]	4.74 ± 0.70 (13)	322 ± 6.8 (4)	152 ± 3.0 (5)
Ca^{++}-free Locke's solution				
Control	16.39 ± 1.12 (6)	4.77 ± 0.87 (6)	316 ± 8.0 (4)	153 ± 5.4 (4)
Acetylcholine + eserine	15.35 ± 2.54 (6)	4.61 ± 1.03 (6)	332 ± 9.8 (4)	150 ± 9.5 (4)

[a] Mean ± standard error.

[b] The number of experiments is indicated in parentheses.

shows that there were no changes either in the tissue orthophosphate and nucleotide contents or in their specific activities under any of the experimental conditions.

In a previous paper it was suggested that a small pool of phosphatidic acid (about 1% of the total phosphatidic acid fraction) should be activated in response to acetylcholine stimulation (2). This suggestion and the fact that a lag period exists between the two responses to acetylcholine stimulation—catecholamine secretion and ^{32}P incorporation into phospholipids (2)—make it unlikely that these two phenomena are causally related. But the most striking feature of the present results, which provide further support for the preceding statement, is that the increased ^{32}P incorporation into phospholipids in response to acetylcholine stimulation was also observed in the absence of Ca^{++}, i.e., under conditions in which, as demonstrated by Douglas and Rubin (7), catecholamine extrusion does not occur.

The increase in ^{32}P incorporation into phospholipids in response to acetylcholine stimulation was of the same magnitude whether or not Ca^{++} was present in the incubation medium. These results disagree with findings in other tissues, in which the observed increases in ^{32}P labeling of phospholipids due to acetylcholine stimulation in Ca^{++}-free medium were 32% and 25% less for phosphatidic acid and phosphatidylinositol, respectively, than in the presence of the cation (8).

The finding that stimulation by acetylcholine, in either the presence or absence of Ca^{++}, did not modify the content of the individual phospholipids of adrenal medullary slices agrees with previous observations on perfused adrenal glands, in which no changes were observed in the lipid content of the adrenal medullae or their different subcellular fractions after stimulation (9–11).

There is no doubt that the effect of acetylcholine increases the turnover of phosphatidic acid in the adrenal medulla, and that phosphatidic acid is an intermediate in the synthesis of phosphatidylinositol in this tissue (2). The experiments described in this paper also clearly demonstrate that there is no correlation between this metabolic effect of acetylcholine and the acetylcholine-evoked catecholamine extrusion.

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