OXYTOCIN DIRECTLY STIMULATES CORTICOSTERONE SECRETION BY DISPERSED RAT ADRENAL ZONAE FASCICULATA AND RETICULARIS CELLS: EVIDENCE FOR THE SPREADING OF THE OXYTOCIN-EVOKED SIGNAL FROM RESPONSIVE TO UNRESPONSIVE CELLS

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ABSTRACT

Oxytocin enhanced basal corticosterone secretion by dispersed rat adrenal zonae fasciculata and reticularis cells, the maximum effect being observed at a concentration 10^{-9} – 10^{-8} M. Video-imaging analysis revealed the existence of a small population of isolated cells (about 5%), responding to the addition of oxytocin (10^{-8} M) by a marked rise in the cytosolic free Ca^{2+} concentration. An interesting phenomenon was also noted, suggesting the propagation of the oxytocin-evoked signal from activated to non-activated cells.

The role of oxytocin in the regulation of adrenocortical function is far from being fully understood (see 4, for review). The presence of oxytocin has been demonstrated in the adrenal glands of many mammalian species, including the rat (1): immunoreactive oxytocin is distributed throughout the entire cortex with the most intense staining in zona glomerulosa, while in the medulla only norepinephrine cells are immunopositive. Such a localization of oxytocin suggests the involvement of this peptide in the regulation of adrenal cortex function. Accordingly, Hinson *et al.* (2) demonstrated a direct stimulatory effect of oxytocin on aldosterone secretion. Here we report findings showing that oxytocin exerts a direct glucocorticoid secretagogue effect on dispersed rat adrenal zonae fasciculata and reticularis cells (inner adrenocortical cells), by activating intracytoplasmatic Ca^{2+} redistribution in a small population of responsive cells.

MATERIALS AND METHODS

The adrenal glands removed from adult female Wistar rats were decapsulated, and isolated inner adrenocortical cells were obtained by sequential

collagenase digestion and mechanical dispersion (8). The viability of isolated cells was checked by the trypan-blue exclusion test and found to be higher than 92%.

Dispersed cells obtained from 6 rats were pooled to obtain a single cell suspension, and 6 cell preparations were employed. Aliquots of each cell suspension (10^5 cells/ml) were incubated with oxytocin (Gedeon-Richter, Budapest, Hungary). The incubation was carried out for 60 min in a shaking bath at 37°C in an atmosphere of 95% O_2 and 5% CO_2 . Corticosterone was measured in the medium by a specific radioimmunoassay (5). Intra- and inter-assay variations were 6% and 8%, respectively.

Other dispersed inner adrenocortical cells were loaded with fura-2, as described by Mason *et al.* (6). Loaded cells in Hepes buffer were transferred into poly-L-lysine-coated measuring vessels placed under a Nikon Diaphot microscope. This inverted microscope was connected to a dynamic video-imaging Magical System (Joyce-Loebel, Gateshead, U.K.). After initial recording of intracellular Ca^{2+} concentration, oxytocin was added at a concentration ranging from 10^{-8} to 10^{-6} M.

Fig. 1 Effect of oxytocin (OX) on basal corticosterone (B) secretion by isolated rat inner adrenocortical cells. Data are means $\pm$ SE ($n = 6$). *, $P < 0.01$ from control group (ANOVA and Multiple Range Test of Duncan)

RESULTS AND DISCUSSION

Oxytocin markedly enhanced basal corticosterone output by dispersed rat inner adrenocortical cells. The effect was already apparent at a concentration 10^{-10} M (53%), and reached its maximum (2-fold) at a concentration 10^{-9} – 10^{-8} M (Fig. 1). Video-imaging analysis revealed the existence of a small population of dispersed cells (about 5%), that responded to oxytocin (10^{-8} – 10^{-7} M) by a rise (about 2-fold) in the cytosolic free- Ca^{2+} concentration. A very interesting phenomenon was also observed: single oxytocin-unresponsive cells were moving with the stream of the medium toward an oxytocin-responsive one; after reaching this cell, the non-activated one began to redistribute intracellular Ca^{2+} achieving the maximum within 30 s (Figs. 2 and 3).

Our present findings clearly indicate that the stimulating effect of oxytocin on corticosterone secretion is due to the direct activation of a small

population of dispersed adrenocortical cells, which in turn is able to propagate the secretagogue signal to the large population of oxytocin-unresponsive cells. The propagation of the oxytocin-induced activation appears to be cell-contact dependent, and this could explain why Hinson *et al.* (2) did not find any effect of oxytocin on corticosterone secretion by collagenase-dispersed rat adrenocortical cells superfused on a Sephadex bed in an isoprene column. It is conceivable that a continuous shaking allows for a continuous transient interaction of suspended cells in the liquid medium, thus leading to the spread of the activation from oxytocin-responsive to oxytocin-unresponsive cells.

Oxytocin stimulates aldosterone secretion by rat zona-glomerulosa cells (2). Hence, we cannot exclude that the small population of oxytocin-responsive cells present in our inner adrenocortical-cell preparations represents zona-glomerulosa cell contamination. However, in our experiments, adrenals have been decapsulated to completely

Fig. 2 Video-imaging of oxytocin (10^{-8} M) effects on the cytosolic free Ca^{2+} concentration in isolated rat inner adrenocortical cells. A single oxytocin non-activated cell is moving with the stream of medium toward the activated one; after reaching activated cell, non-activated one begins to redistribute intracellular Ca^{2+} .

remove zona glomerulosa (9), and zona-glomerulosa cells are markedly smaller than inner adrenocortical ones (7), which are very similar to the fluorescent (i.e. oxytocin-responsive) cells of our preparations.

The physiological meaning of our present results remains to be investigated. However, we want to recall that it has been recently shown (3) that physiological concentrations of oxytocin stimulate ACTH secretion by pituitary corticotropes, by mobilizing Ca^{2+} mainly from intracellular stores.

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Fig. 3 Effect of oxytocin (OX) (10^{-8} M) on Ca^{2+} redistribution in isolated rat inner adrenocortical cells. Video-imaging record is shown (upper panel), which refers to the oxytocin-responsive (1) and oxytocin-unresponsive (2) cells, visualized in the lower panel (A and B). The record demonstrates that the unresponsive cell displays Ca^{2+} redistribution upon reaching contact with the responsive one.

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