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Dissociation of Alpha-adrenergic and Cholinergic Stimulated Inositol Trisphosphate-Dependent Calcium Mobilization at the "Post-Receptor" Level

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Carbachol stimulated inositol trisphosphate (IP_3) production and subsequent calcium mobilization in parotid cells are almost completely inhibited by neomycin. In contrast epinephrine stimulated IP_3 production and calcium mobilization are much less sensitive to such inhibition. Since neomycin exerts its effects primarily at the level of inositol phosphate production and action, cholinergic and alpha adrenergic stimulation of IP_3 dependent calcium mobilization may proceed through different "post-receptor" signal transduction mechanisms in parotid cells.

$Alpha_1$-adrenergic(1,2) and muscarinic cholinergic(2-4) agents have been shown to stimulate parotid cell secretion through an inositol-1, 4, 5-trisphosphate (IP_3) and calcium dependent mechanism. Although these agents interact with separate cell surface receptors, their subsequent activation of phospholipase C, and IP_3 and calcium stimulated signal transduction events, are believed to be quite similar in most cell types(1,2,5,6). However, several studies have suggested that the "post-receptor" portions of the alpha-adrenergic and muscarinic cholinergic pathways might be dissociable(7-9).

For example, alpha-adrenergic and exogenous IP_3 stimulated calcium mobilization decline 40-50% during aging, while cholinergic stimulated calcium mobilization is reduced by only 20% (8,9). No age differences in stimulated levels of IP_3 occur, thus suggesting possible differences in the intracellular location in the IP_3 generated by different agents (as well as exogenously administered IP_3) at different ages(9).

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Moreover, some recent work has suggested that adrenergic and cholinergic agents may additionally mediate changes in calcium flux through second messengers other than IP_3 (e.g. arachindonate, prostaglandins and other factors) (7,10-12). In light of the observed age differences in adrenergic and cholinergic action, such messengers might influence calcium movement in both positive and negative ways and change differentially with age. The present study was, therefore, designed to determine whether $alpha_1$-adrenergic and cholinergic agonists stimulate calcium mobilization through different signal transduction mechanisms, and if so, at what point(s) their respective sequences diverge.

Materials and Methods

Parotid cell aggregates were prepared from 3 month old male Wistar rats by collagenase/hyaluronidase digestion as previously described.(9) Cells were labeled with ^{45}Calcium (New England Nuclear) for 60 minutes, washed in Hanks Balanced Salt Solution and exposed to various concentrations of neomycin sulfate or buffer only for 30 minutes prior to administration of 5×10^{-5} or 10^{-6}M epinephrine or 10^{-5}M carbachol. Previous studies in our laboratory have shown that maximal stimulation occurs in the presence of 5×10^{-5}M epinephrine (9,13) and 10^{-5}M carbachol (8) and that the epinephrine response is completely mediated through the $alpha_1$ adrenergic receptor subtype (14). ^{45}Calcium release into the medium was measured after 1 minute as previously described.(9)

For IP_3 measurement, parotid cells were prepared and exposed to epinephrine, carbachol, and/or neomycin as above, except that the ^{45}Ca labeling step was omitted. After one minute of stimulation cells were mixed with 20% perchloric acid and kept on ice for 20 min before centrifugation. The supernates were then neutralized with 5M KOH-0.5M HEPES and recentrifuged. 100µl aliquots were used in the D-myo-inositol 1, 4, 5-trisphosphate assay system (Amersham).

Results

Neomycin has been reported to inhibit both IP_3 action and production by binding to inositol phosphates as well as inhibiting phospholipase C(15,16). In order to determine whether these signal transduction events might be qualitatively and/or qualitatively different in response to alpha-adrenergic and cholinergic stimulation, subsequent calcium mobilization was examined in the absence and presence of the drug. Maximal ^{45}Ca efflux stimulated by both 5×10^{-5}M epinephrine and 10^{-5}M carbachol was about 10% of the total cellular content following a 1 hr labeling period (Fig. 1). Increasing concentrations of neomycin completely abolished the response to 10^{-5}M carbachol, without significantly affecting the response to 5×10^{-5}M epinephrine.

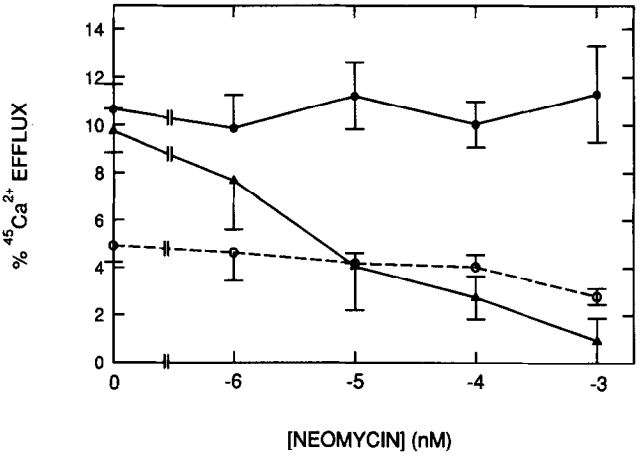

Fig. 1. Effect of neomycin on epinephrine and carbachol stimulated ^{45}Ca efflux in rat parotid cells. Values represent the means ± standard errors of 3-6 separate experiments in which all concentrations of carbachol, epinephrine and neomycin were administered in triplicate. ●, 5 X 10^{-5}M epinephrine; ○, 10^{-6}M epinephrine; ▲, 10^{-5}M carbachol.

Fig. 2 shows that neomycin almost completely inhibited 10^{-5}M carbachol stimulated IP_3 production while only reducing 5 X 10^{-5}M epinephrine stimulated IP_3 production by about half. However, maximal stimulation of IP_3 production was about twice as great for epinephrine as for carbachol. Thus, the amount of IP_3 generated in the presence of maximal epinephrine plus neomycin was approximately equal to that generated by maximal carbachol in the absence of neomycin. It was therefore possible that this level of IP_3 is sufficient to stimulate maximal (~ 10%) calcium efflux and that differences between epinephrine and carbachol in neomycin inhibition of calcium efflux

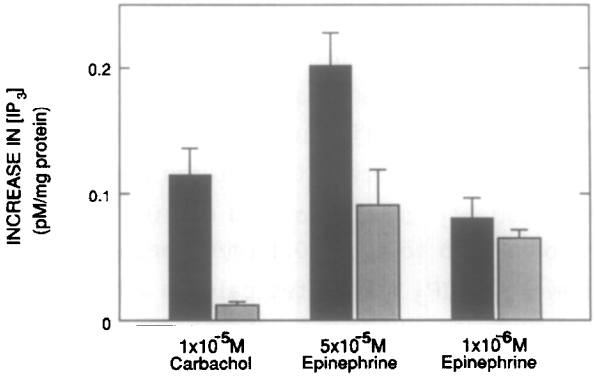

Fig. 2. Effect of neomycin on epinephrine and carbachol stimulated IP_3 generation. Values represent the means of 3-5 separate experiments in which all concentrations of carbachol, epinephrine and neomycin were administered in triplicate. Unstimulated levels of IP_3 were 0.17 ± 0.01 pm/mg protein. Solid bars represent the absence of neomycin, while dotted bars indicate the presence of neomycin.

were simply due to the greater stimulation of IP_3 production by epinephrine.

To investigate this possibility 10^{-6}M epinephrine (which had been previously shown in our laboratory to stimulate IP_3 production approximately half maximally(9) was administered. Fig. 2 confirms the approximately half maximal effectiveness of this concentration of epinephrine and also shows that neomycin only slightly reduced IP_3 production under these conditions. At the same time, Fig. 1 shows that 10^{-6}M epinephrine is also approximately half maximal for stimulation of calcium efflux. Neomycin inhibits this response only 20-40%. Thus, even at a concentration of epinephrine which generates roughly the same amount of IP_3 as maximal carbachol, inhibition of calcium mobilization by neomycin is much less for epinephrine than for carbachol.

Discussion

These data suggest that qualitative and/or quantitative differences exist in the mechanism(s) by which alpha-adrenergic and cholinergic agonists stimulate IP_3 production. It is not known whether separate phospholipase C enzymes and guanine nucleotide sensitive coupling proteins (G proteins) exist for the 2 systems. Preliminary experiments suggested that epinephrine and carbachol stimulated calcium mobilization were equally inhibited ($\sim$ 30%) in parotid cells pretreated for 2 hrs with 4 µg/ml pertussis toxin. These experiments imply G protein involvement but provide no evidence for dissociation of adrenergic and cholinergic signal transduction at this level. Since present results reveal differences in neomycin sensitivity for IP_3 production and subsequent calcium flux, it seems more likely that epinephrine and carbachol may activate different phospholipase C enzymes or differentially activate the same enzyme. Although a roughly linear correlation between epinephrine and carbachol stimulated IP_3 production and calcium efflux exists for concentrations of IP_3 up to about 0.1 pm/mg protein, differential effects of neomycin on IP_3 stimulated calcium mobilization (possibly due to differences in intracellular compartmentalization) cannot be ruled out.

Finally, it is also quite possible that stimulation of second messengers other than IP_3 by epinephrine and carbachol may result in differential modulation of phospholipase C activity and/or calcium

mobilization. Such mechanisms would also help to explain how responsiveness to adrenergic and cholinergic agonists is differentially affected by aging (8, 9). Although a few such messengers have already been identified [e.g., arachidonate(10), prostaglandins(7), guanosine triphosphate(11), and inositol tetrakisphosphate(12)] whether these or other similar agents truly play a role in regulating alpha-adrenergic and cholinergic stimulated calcium mobilization remains to be determined.

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