Forskolin as a tool to study the β-adrenergic receptor-elicited, labeled protein secretion in rat lacrimal gland

Philippe Mauduit, Geneviève Herman and Bernard Rossignol

Laboratoire de Biochimie des Transports Cellulaires, Institut de Biochimie, Bât 432, Université de Paris-Sud, 91405 Orsay Cedex, France

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In rat lacrimal glands, Forskolin induces a dose-dependent [^{3}H]protein release. This effect can be potentiated by papaverine. As for the other inducers whose effects on protein secretion are assumed to be cAMP-mediated, Forskolin secretion time course shows a latency. Isoproterenol decreases the Forskolin EC_{50} at least 60-times. On the other hand, Forskolin enhances the efficacy of isoproterenol without affecting its potency. As a whole, the data collected show that isoproterenol-induced [^{3}H]protein secretion in rat lacrimal glands involved adenylate cyclase activation by coupling with β-adrenergic receptors.

Forskolin *β-Adrenergic receptor* *Protein secretion* *Rat lacrimal gland*

1. INTRODUCTION

Some studies have shown the possibility of inducing a large peroxidase [1] or labeled protein [2] secretion via β-adrenergic receptors in rat lacrimal glands. In our laboratory, we found that this stimulation was accompanied by an increase in tissue cAMP content (submitted). On the other hand, authors in [3] failed to elicit a labeled protein secretion through a β-adrenergic receptor activation. However, the same authors have described the possibility of promoting a significant labeled protein discharge via ACTH and/or α-MSH receptors coupled with an adenylate cyclase activity [4].

Forskolin, a diterpene, was shown to directly and specifically activate the adenylate cyclase in membrane preparations as well as in intact cells from various tissues [5,6]. Up to now, only a few of these studies have been performed on physiological processes dependent (or postulated to be) on an intracellular cAMP level increase due to an adenylate cyclase activation [7–9].

Recently, it has been reported that Forskolin activates the fluid secretion in blowfly salivary glands [10]. Thus, Forskolin appears to be a useful tool to investigate whether isoproterenol induced labeled protein secretion, in rat lacrimal glands, is elicited by a mechanism involving a coupling between β-adrenergic receptors and adenylate cyclase activity. This work shows that Forskolin evokes a labeled protein secretion and that it enhances the isoproterenol stimulation by increasing the efficacy of β-adrenergic agonist without affecting its potency.

Abbreviations: PGE_1, PGE_2, PGD_2 and PGI_2, prostaglandin E_1, E_2, D_2, I_2; cAMP, 3′,5′-adenosine monophosphate; ACTH, adrenocorticotropic hormone; α-MSH, α-melanocyte-stimulating hormone; VIP, vasoactive intestinal peptide; TSH, thyroid-stimulating hormone

2. MATERIALS AND METHODS

2.1. *Chemicals*

L-[^{3}H]Leucine was purchased from the C.E.A. Saclay, France. L-Isoproterenol bitartrate and papaverine hydrochloride were from Sigma, St Louis. Atropine sulfate was purchased from Calbiochem (Los Angeles CA), l-propranolol from ICI Pharmacia (Enghien) and phentolamine

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sulfonate from Ciba Geigy Laboratories (Lyon). Forskolin was a kind gift from Hoechst France and was made up as a 10 mM stock solution in 75% ethanol (v/v).

2.2. *Biological material*

Fragments of lacrimal glands from male albino Sprague–Dawley rats (6–8 weeks old) were prepared as in [11].

2.3. *Incubation procedures*

2.3.1. 'Fixed time' discharge experiments

Incubation procedure, pulse labeling (10 min) and protein discharge were performed as in [11,12]. The protein pellet was dissolved in 0.5 N NaOH and the radioactivity was determined using aqualuma as scintillation cocktail on Packard Tri-Carb Scintillation Counter.

Results were expressed either directly as the amount of labeled proteins present in the incubation medium, as a percentage of total labeled proteins in tissue and medium, or by the stimulation factor which is the ratio of the % of protein secreted under stimulation to the % of protein secreted in the unstimulated state at the same time.

2.3.2. Time course of protein secretion

Incubation procedure and pulse labeling were as above, but protein discharge was determined on a duplicate aliquot (0.5 ml) of the incubation medium for each time indicated in legends. The protein radioactivity of both tissue and medium was determined as above. The results were expressed as cumulative protein secretion *vs* time.

All [^{3}H]protein discharges were performed in the presence of the same amount of ethanol (0.75% final concentration) to avoid a possible effect of the Forskolin solvent on the secretory process. Each experiment was performed 3-times and each result reported is a representative one.

3. RESULTS

The effects of Forskolin on protein secretion in rat lacrimal glands pieces are shown in fig.1. Forskolin alone induced a concentration-dependent release of labeled proteins; this effect could be potentiated by 10 μM papaverine, a cAMP phosphodiesterase inhibitor (fig.1A). The Forskolin stimulation was not affected by the antagonists of muscarinic or adrenergic receptors (atropine, phentolamine or l-propranolol; not shown). Fig.1B shows the effect of isoproterenol (a specific β-adrenergic agonist) on labeled protein secretion induced by increasing concentrations of Forskolin. Since the dose–response curve for Forskolin alone failed to reach any plateau in the concentration range tested, half the maximal effect (EC_{50}) can only be estimated to be higher than 30 μM Forskolin. Isoproterenol at 5 μM shifted this EC_{50} to about 0.5 μM. At 10 μM Forskolin, isoproterenol substantially increases protein secretion, but at 100 μM, it was almost the same as the theoretical sum of the two individual responses (Forskolin or isoproterenol alone). The effect of 3 μM Forskolin which elicited only a partial stimulation of protein secretion was tested on the secretory response due to increasing concentrations of isoproterenol. The data in fig.2 show that, while this limited concentration of Forskolin did not significantly affect the EC_{50}-value for isoproterenol, it increased its maximal response to a level higher than the sum of the two individual responses.

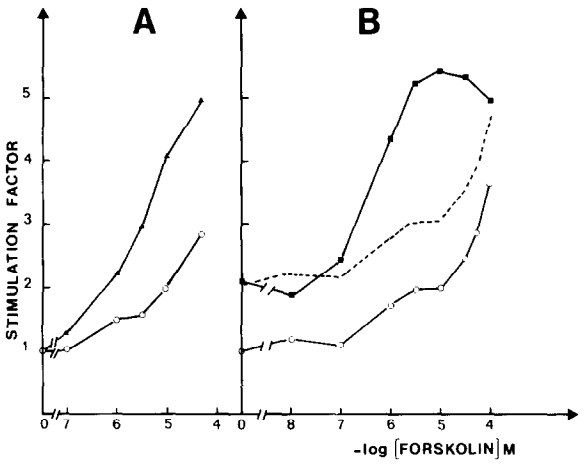

Fig.1. Effect of papaverine and isoproterenol on Forskolin-induced protein secretion. [^{3}H]protein discharge was performed in the presence of various concentrations of Forskolin for 40 min. (A) Incubations were performed in the absence (○—○) or in the presence (▲—▲) of 10 μM papaverine added 10 min before Forskolin; (B) Incubations in the absence (○—○) or in the presence (■—■) of 5 μM isoproterenol added along with Forskolin. Dashed line indicates the theoretical sum of the two individual responses for each Forskolin concentration.

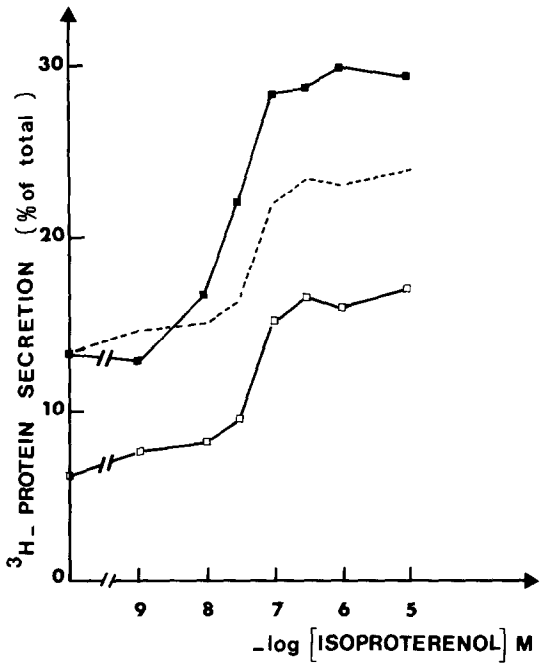

Fig.2. Effect of Forskolin on isoproterenol-induced protein secretion. [^{3}H]protein secretion was performed in the presence of various concentrations of isoproterenol both in the absence (□—□) or in the presence (■—■) of 3 μM Forskolin. Dashed line indicates the theoretical sum of the two individual responses for each isoproterenol concentration.

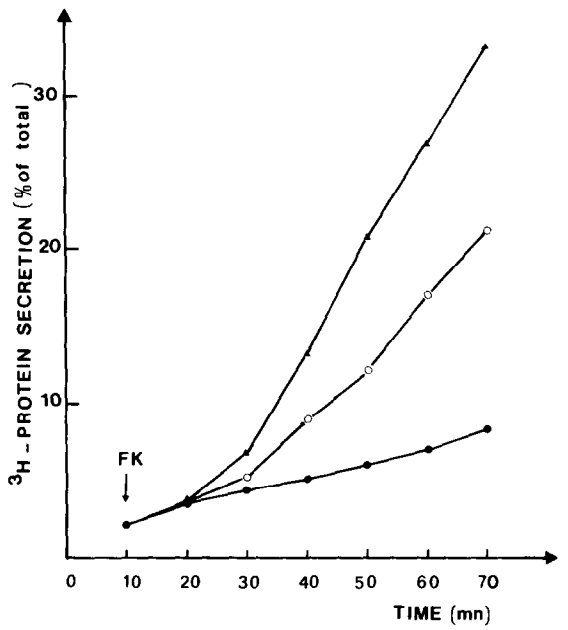

Fig.3. Time course of protein secretion elicited by Forskolin. Time course of protein secretion was performed as indicated in section 2. Incubations were conducted in the absence (○—○) or in the presence (▲—▲) of 10 μM papaverine added 10 min before 10 μM Forskolin. (●—●) indicates unstimulated protein secretion both in the absence or presence of papaverine.

The time course of labeled protein secretion under 10 μM Forskolin stimulation in the presence or absence of papaverine, as shown in fig.3, demonstrates the existence of a lag time period. In both cases the linearity of the protein secretion was obtained 20 min after Forskolin was added to the incubation medium. Papaverine, which has no effect by itself, promotes a 2-fold stimulation of the net secretion rate induced by Forskolin.

4. DISCUSSION

Here we used Forskolin, a potent activator of adenylate cyclase in order to have an indirect proof of cAMP involvement in the regulation of the exocrine secretory process in rat lacrimal glands, mainly through the coupling β-adrenergic receptor-adenylate cyclase. It was shown that Forskolin alone was able to elicit a significant protein secretion as soon as 1 μM (fig.1A), a concentration which provokes a 50% maximal increase in cAMP accumulation in blowfly salivary glands as in [10]. Moreover, as could be postulated for a cAMP-dependent mechanism, an agent which inhibits the cAMP degradation (papaverine) increases the secretory response for all the Forskolin concentrations tested. A similar result was obtained for cAMP accumulation in rat brain slices [13] with ZK 62771 as phosphodiesterase inhibitor. It is noteworthy that the protein discharge induced by Forskolin cannot be due to receptor activation or result from neurotransmitter release from nerve endings since none of the antagonists of the muscarinic, α- and β-adrenergic receptors present in the lacrimal gland was able to inhibit the secretory response to the diterpene.

The effect of isoproterenol on the response to increasing concentrations of Forskolin, i.e., the shift in the EC_{50} concentration of Forskolin (from more than 30 μM to 0.5 μM), is analogous to the results reported with PGE_1 on Forskolin stimulation of adenylate cyclase activity in membrane prepara-

tions [14] or with PGE_1, PGD_2, PGI_2, isoproterenol and TSH on cAMP accumulation in intact cells [7,8,15].

The isoproterenol-induced stimulation of protein discharge is potentiated by low concentration of Forskolin since the maximal secretory response in the presence of both products is higher than the sum of the response for the products alone. The efficacy of isoproterenol is increased but its EC_{50} does not seem to be shifted by the diterpene. This result agrees with those obtained with the stimulation of cAMP accumulation by histamine in guinea-pig brain slices, norepinephrine in rat brain slices [6] and TSH in bovine thyroid glands. However, our result is different from those where Forskolin increased only the potency of the agonists, VIP and PGE_2 [6], or where it increased both the efficacy and the potency of the agonists PGE_1, PGD_2 and PGI_2 [7]. Moreover, our data can also be compared to those reported for other physiological processes: the effect of Forskolin on steroidogenesis induced by ACTH [9], on lipolysis induced by isoproterenol [8] and on the inhibition of platelet aggregation induced by PGE_1 [7].

The absence of labeled protein detected in the incubation medium above that of the control for 10 min after Forskolin has been added, was in agreement with the latency in the time course of peroxidase [1] or labeled protein secretion (submitted) elicited in the same tissue by inducers whose effects are assumed to be cAMP-mediated: isoproterenol, db-cAMP or methylxanthines. On the other hand, the authors in [3,4] did not report a latency concerning the labeled protein discharge induced in rat lacrimal glands by ACTH nor by α-MSH, even though they show that these responses are cAMP-mediated. This discrepancy between the ACTH or α-MSH responses and the others also postulated to cAMP-mediated has not been elucidated.

The results obtained with Forskolin on the stimulation of the [^{3}H]protein discharge in rat lacrimal glands are well correlated to those reported for the adenylate cyclase activity or cAMP accumulation increased by this diterpene in other tissues.

As opposed to the results in [3,4], the data reported here suggest that labeled protein secretion can be triggered off in rat lacrimal glands through a mechanism involving a cAMP intracellular level increase (submitted) due to adenylate cyclase activation by coupling this enzyme with β-adrenergic receptors.

Furthermore, Forskolin appears to be a very useful tool in defining the role of adenylate cyclase activity in the hormonal or neurotransmitter regulation of protein secretion in exocrine glands.

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REFERENCES

[1] Friedman, Z.Y., Lowe, M. and Zeliger, Z. (1981) Biochim. Biophys. Acta 675, 40–45.
[2] Mauduit, P., Herman, G. and Rossignol, B. (1982) Biol. Cell 45, 281.
[3] Jahn, R. and Söling, H.D. (1981) FEBS Lett. 131, 28–30.
[4] Jahn, R., Padel, U., Porsch, P.H. and Söling, H.D. (1982) Eur. J. Biochem. 126, 623–629.
[5] Seamon, K.B., Padgett, W. and Daly, J.W. (1981) Proc. Natl. Acad. Sci. USA 78, 3363–3367.
[6] Seamon, K.B. and Daly, J.W. (1981) J. Cyclic Nucleotide Res. 7, 201–224.
[7] Siegl, A.M., Daly, J.W. and Smith, J.B. (1982) Mol. Pharmacol. 21, 680–687.
[8] Litosh, I., Hudson, T.H., Mills, I., Shih-Ying Li and Fain, J.N. (1982) Mol. Pharmacol. 22, 109–115.
[9] Moriwaki, K., Itoh, Y., Iida, S. and Ichihara, K. (1982) Life Sci. 30, 2235–2240.
[10] Litosh, I., Saito, Y. and Fain, J.N. (1981) Biochem. J. 204, 147–151.
[11] Chambaut-Guerin, A.M., Muller, P. and Rossignol, B. (1978) J. Biol. Chem. 253, 3870–3876.
[12] Rossignol, B., Herman, G. and Clauser, H. (1969) Biochem. Biophys. Res. Commun. 34, 111–119.
[13] Daly, J.W., Padgett, W. and Seamon, K.B. (1982) J. Neurochem. 38, 532–544.
[14] Insel, P.A., Stenge, D., Ferry, N. and Hanoune, J. (1982) J. Biol. Chem. 257, 7485–7490.
[15] Fradkin, J.E., Cook, G.H., Hilhoffer, M.C. and Wolff, J. (1982) Endocrinology 111, 849–856.