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Adenylate cyclase activity of olfactory bulb and epithelium of the goat

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SUMMARY - We have partially characterized the adenylate cyclase activity in the goat olfactory bulb, epithelium and cilia. In all preparations the enzyme activity was dually regulated by GTP and sensitive to GTP analogue, GTPγS, and fluoride ions. Olfactory cilia exhibited higher levels of adenylate cyclase activity than the whole epithelium. GTPγS, fluoride ions and forskolin greatly enhanced the cilia enzyme activity. Odorants classified as floral, fruity and minty slightly stimulated adenylate cyclase: 116-136% above the basal level. The findings reported here suggest that in the goat olfactory epithelium an adenylate cyclase qualified for the transduction of olfactory signals is present.

Key words: Adenylate cyclase, cAMP, olfactory cilia, G protein activators, odorants.

RESUME - "Activité de l'adénylcyclase dans le bulbe et l'épithélium olfactifs de la chèvre". Nous avons partiellement caractérisé l'activité de l'adénylcyclase dans le bulbe, l'épithélium et les cilia olfactifs de la chèvre. Dans toutes les préparations l'activité de l'enzyme était dualement régulée par le GTP et elle était sensible à l'analogue du GTP (GTPγS) et aux ions fluorure. Les cilia olfactifs ont montré des niveaux plus hauts de stimulation de l'adénylcyclase que l'épithélium entier. GTPγS, les ions fluorure et forskolin ont augmenté l'activité enzymatique des cilia. Les odorants de la classe florale, des fruits et de la menthe ont stimulé l'adénylcyclase de 116-136% par rapport au niveau de base. Ces résultats suggèrent la présence d'un phénomène de transduction qui semble être lié au système de l'adénylcyclase dans l'épithélium olfactif de la chèvre.

***Mots clés** : Adénylcyclase, AMP cyclique, cilia olfactifs, activateurs de la protéine G, odorants.*

Introduction

The sense of smell is carried out by olfactory receptors that lie deep within the nasal cavity, distributed into the olfactory epithelium. This epithelium contains three types of cells: receptors, supporting and basal cells. The receptors are bipolar neurons extending an axon into the olfactory bulb of the brain, and a peripheral process toward the nasal lumen. Such peripheral process expands into a small knob that gives rise to several cilia that are thought to interact with the odour producing molecules. The longer central process runs to the bulb together with many other axons, forming the olfactory nerve (Getchell, 1986; Anholt, 1991).

Odorants stimulate olfactory cells by first being absorbed into the mucous layer and bound to a specific protein carrier (Bignatti *et al.*, 1988). The molecule then diffuses to the cilia of the receptor neurons and binds to sites specific for shape and size. This event initiates a multistep reaction cascade, leading to the opening of cationic channels on the plasma membrane, the subsequent depolarization of the receptor cells and the final action potential on the chemosensory neuron (Getchell, 1986). Supporting and basal cells can respond to odour stimulation showing a small depolarization, probably connected with the releasing of mucus. Several lines of evidence support the notion that, after the interaction odorant-receptor takes place, a transduction system is responsible for the coupling between the extracellular signal and the intracellular depolarization. As a matter of fact, the olfactory receptors respond to the binding of an odorant molecule by changing the rate of synthesis of an intracellular second messenger, such as adenosine 3',5'-monophosphate (cAMP) or inositol triphosphate (IP3) and diacylglycerol (DAG). By activating specific protein kinases, they induce phosphorylation of several membrane proteins that modulate the opening or the closure of ion channels (Firestein, 1991; Vodyanoy, 1991). It has also been suggested a direct interaction of cAMP with the cyclic nucleotide-gated ion channel thus providing a mechanism for rapid electrical signalling (Nakamura and Gold, 1987; Dhallan *et al.*, 1990). A given odorant molecule seems to selectively influence the synthesis of only one second messenger (Breer and Boekhoff, 1991).

As for cAMP synthesizing enzyme, the adenylate cyclase, its role in olfactory transduction has been well established (Pace *et al.*, 1985; Lowe *et al.*, 1989). This enzymatic complex consists of (i) a receptor, that binds the odorant molecule; (ii) a catalytic subunit, responsible for the conversion of ATP to cAMP; and (iii) a coupling component, called G protein because of its affinity for guanine nucleotides (Gilman, 1987). G proteins are a group of homologous proteins which share a common heterotrimeric structure. Although they show many similar properties, they also exhibit subtle sequence differences that may provide a mechanism by which a limited number of signal transductors can modulate different pathways (Strathmann and Simon, 1990). The involvement of G proteins in olfaction has been suggested in fish, amphibia and mammals (Reed, 1990). As in other tissues, several G proteins have been identified in the olfactory epithelium, where a novel G protein, designed as G olf, has been also demonstrated. G olf is a stimulator protein that, activated by odorants, positively affects the adenylate cyclase catalytic unit (Jones and Reed, 1989).

An odorant molecule could influence the adenylate cyclase just like a neurotransmitter or an hormone: its binding to the receptor may induce the exchange GDP/GTP on the alpha subunit of G proteins and dissociation from the trimeric complex of the alpha subunit which, in turn, activates the enzyme. Hydrolysis of GTP by an intrinsic enzymatic activity returns the subunits to their original conformations (Gilman, 1987).

Since not very much information is available about the olfactory transduction in non rodents mammals, the aim of this work is to reveal the presence of an adenylate cyclase activity in different preparations from goat olfactory epithelium, as well as in the olfactory bulb. Moreover, we describe the behaviour of such enzyme in different experimental conditions, together with its responsiveness to physiological or non physiological ligands, as well as to odorant molecules.

Methods and materials

Tissue preparation

After the nasal cavity was dissected open, the olfactory epithelium with the underlying cartilage and the olfactory bulb were removed.

Olfactory cilia: sheets of olfactory epithelia were obtained and placed in ice-cold Ringer's solution following the procedure reported by Anholt *et al.* (1986) and Chen *et al.* (1986) with slight modifications. Cilia were detached by gently stirring (20 min at 4°C) the tissue in Ringer's solution supplemented with 10 mM $CaCl_2$. The sample was centrifuged for 15 min at 6000 g: the pellet was again processed for the deciliation while the supernatant, containing cilia, was kept on ice. The final pellet was used for the preparation of deciliated epithelium. The supernatants obtained from the two steps were centrifuged at 12000 g for 15 min and the pellet washed two times with 10 mM Tris-HCl, pH 8.0, containing 3 mM $MgCl_2$ and 2 mM EDTA.

Deciliated epithelium: the 6000 g centrifugation pellet was homogenized in 10 mM Tris-HCl, pH 8.0, and centrifuged at 500 g for 10 min. Supernatant aliquots were used for adenylate cyclase assays.

Whole epithelium and olfactory bulb: the tissues were homogenized in 10 mM Tris-HCl, pH 8.0, and centrifuged at 500 g for 10 min. Supernatant aliquots were used for adenylate cyclase assays.

Assay of adenylate cyclase activity

Adenylate cyclase (E.C.4.6.1.1.) activity was measured according to Clement-Cormier *et al.* (1975) with slight modifications. 150-200 μg proteins of tissue preparations were preincubated for 20 min at 0°C in a final volume of 400 μl containing: 80.2 mM Tris-Maleate pH 7.4 buffer, 2 mM $MgSO_4$, 0.6 mM EGTA, 0.5 mM GTP, 10 mM aminophylline and test substances. Odorants were dissolved in Lamacit ER (PEG 20 glyceril ricinoleate plus PEG 40 ricinoleamide). The reaction was initiated by the addition of 0.5 mM ATP. The incubation was carried out in a shaking bath for 5 min at 30°C and stopped by immersing the tubes in boiling water for 2 min. The samples were then frozen at -20°C.

cAMP determination

The samples were thawed and centrifuged at 2000 g for 10 min; aliquots of clear supernatants were used for cAMP determination according to the radiochemical method of Brown *et al.* (1972). Adenylate cyclase specific activity is expressed as moles cAMP mg $protein^{-1}$ 5 min^{-1}.

Protein determination

Protein levels were measured as reported by Lowry *et al.* (1951) using bovine serum albumin as standard.

Materials

3H-cAMP (Sp.Act.28Ci/mmol) was obtained from the Radiochemical Centre, Amersham, UK. ATP, GTP and Forskolin were purchased from Calbiochem, La Jolla, CA, USA; cAMP and GTPγS from Boheringer Mannheim GmbH, Milano, Italy. Odorants were kindly provided by Prof. G.P. Pollini, Dept. of Pharmaceutical Chemistry, University of Ferrara, Italy. Other chemicals were the highest reagent grade commercially available.

Results and discussion

As a first step, we tested the time-dependence of basal adenylate cyclase activity in different preparations obtained from goat olfactory epithelium and bulb.
As shown in Fig. 1, the enzyme activities increase with the incubation time, but they don't reach a plateau, at least up to 30 min. In all assays, the bulb preparation exhibits the highest enzymatic activity, followed by the whole epithelium and finally by the deciliated one. For the subsequent experiments, an incubation time of 5 min was chosen.

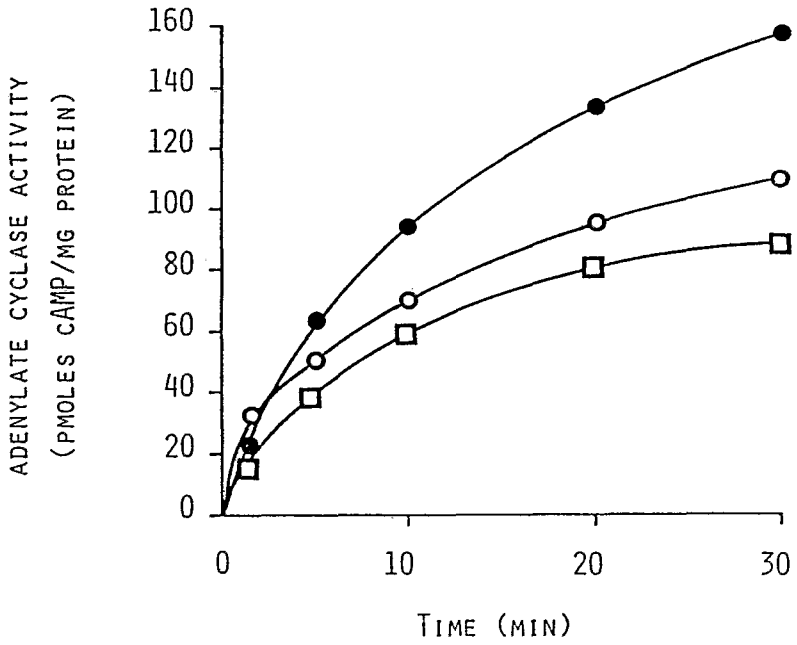

Fig. 1. Time course of adenylate cyclase activity from goat olfactory bulb (●-●), whole epithelium (O-O) and deciliated epithelium (□-□).

The dependence of adenylate cyclase activity on protein concentration has been then tested on the same preparations. As shown in Fig. 2, cAMP synthesis is proportional to protein content up to 400 g per assay for bulb and whole epithelium preparations, and up to 300 g per assay for deciliated epithelium. For the following experiments, a protein concentration of about 200 g per sample was used.

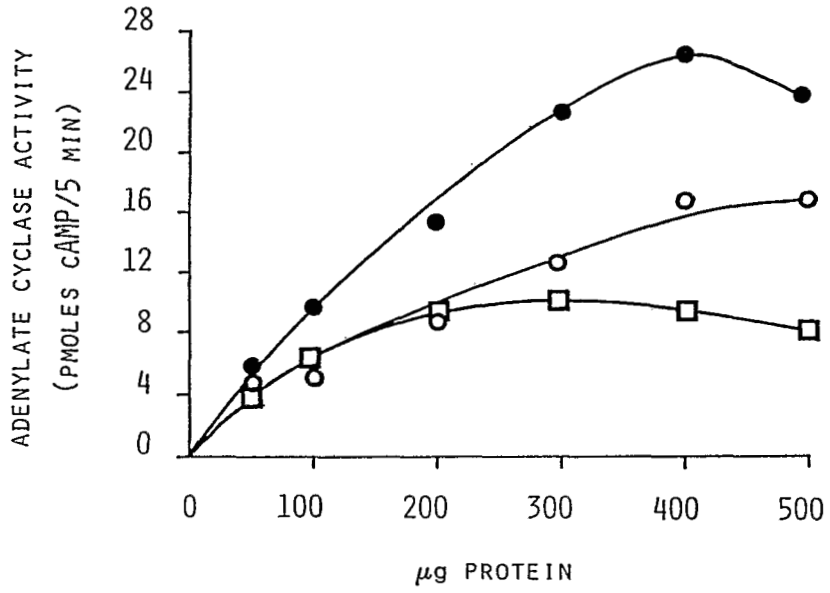

Fig. 2. Dependence of adenylate cyclase activity on protein concentration in goat olfactory bulb (●-●), whole epithelium (O-O) and deciliated epithelium (□-□).

It is well known that receptorial modulation of adenylate cyclase is carried out through the guanine nucleotide binding regulatory proteins, Gs and Gi. Thus we investigated the GTP dependency of the enzyme activity in our preparations. As shown in Fig. 3, in all cases lower nucleotide concentrations (up to 10 μM) stimulate the enzyme activities which, in the absence of GTP, are 58.1 ± 5.0 moles cAMP mg protein^{-1} 5 min^{-1} in bulb, 38.6 ± 4.0 moles cAMP mg protein^{-1} 5 min^{-1} in whole olfactory epithelium and 36.1 ± 3.9 moles cAMP mg protein^{-1} 5 min^{-1} in deciliated epithelium. In the presence of higher nucleotide doses, a decline in enzymatic activities is evident.

Different experimental approaches indicate that the odorant-activated adenylate cyclase is mainly localized in ciliary membranes (Pace *et al.*, 1985). Thus we studied the behaviour of the enzyme activity from goat cilia in different experimental conditions. The time-course is reported in Fig. 4. As shown, also in this preparation the enzyme activity increases with time without reaching a plateau. The GTP dependence (Fig. 5) is similar to that just described for the olfactory bulb and epithelia, though the modulatory action of GTP is more pronounced in the cilia than in epithelia. In addition, the basal adenylate cyclase activity from cilia is higher than that from the whole as well as the deciliated epithelium, both in the absence or in the presence of GTP. The bimodal effect of the nucleotide is observed in almost all the examined

tissues and it is explained on the basis of different affinities of GTP for the guanine nucleotide binding proteins (Arima *et al.*, 1986; Biondi *et al.*, 1990). At lower GTP concentrations the dissociation of the stimulator G protein is favoured, therefore inducing an enhancement of adenylate cyclase activity. At higher doses of the nucleotide, the dissociation of the inhibitory G protein becomes significant and a reduction of the enzymatic activity is observed. Thus in our experimental preparations, G proteins are at least of two different kinds: (i) the stimulator G olf, whose activation is induced by lower GTP concentrations; (ii) the inhibitory Gi, activated by higher nucleotide doses, mediating the reduction of the adenylate cyclase activity.

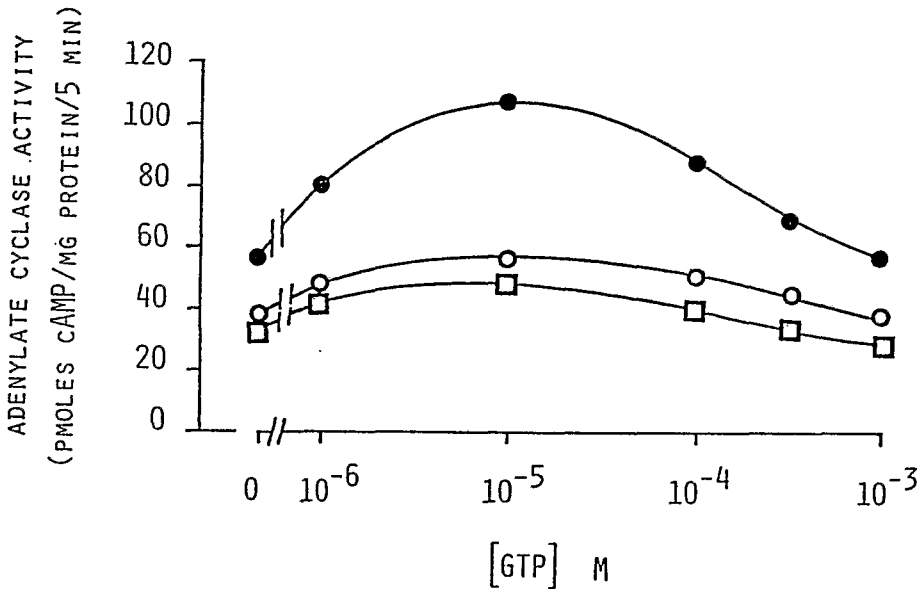

Fig. 3. Effect of increasing concentrations of GTP on adenylate cyclase activity from goat olfactory bulb (●-●), whole epithelium (O-O) and deciliated epithelium (□-□).

In almost all tissues, the adenylate cyclase activity is modulated by non physiological agents interacting with G proteins and/or the catalytic moiety of the enzyme. Among these substances are the non-hydrolysable analogues of GTP, such as 5'-0-(3'thiophosphate) (GTPγS), that compete with the endogenous guanine nucleotide for the same site on G proteins. Due to their slow dissociation from the activated G proteins (Asano *et al.*, 1984), these analogues allow a persistently elevated adenylate cyclase activity. A similar effect is induced by fluoride ions, which interact with G proteins by mimicking the γ-phosphate of GTP (Bigay *et al.*, 1987). Moreover the diterpene forskolin activates the adenylate cyclase from different tissues, interacting mainly with the catalytic subunit of the enzyme (Seamon, 1987). Thus, all these substances are useful tools for evaluating the responsiveness of both G proteins and the catalytic unit of the enzymatic complex.

In Table 1 and in Fig. 6 are illustrated the effects of 10 μM GTPγS, 10 mM fluoride ions and 10 μM forskolin on cilia and bulb, as well as on olfactory epithelia preparations. The stimulator effect of GTPγS is more pronounced in cilia and bulb

(about 250%) than in other preparations; as for fluoride ions, a 2-3 fold stimulation is observed in bulb and epithelia, while a 6-fold activation is obtained in cilia. The latter preparation is also highly responsive (467%) to forskolin. In Fig. 6 the effect of some odorant molecules on the adenylate cyclase from goat olfactory cilia is also described. As shown, these substances have no effect or induce only slight stimulations of the enzyme activity. These results are not in line with those obtained in other animals, mainly amphibia and rodents, where similar molecules activate more efficaciously the adenylate cyclase of olfactory cilia (Sklar *et al.*, 1986; Coon *et al.*, 1989). Such discrepancy could be explained assuming that different odorant molecules are effective in the different animal species. Moreover, it is difficult to test the odorant effect in an aqueous medium because of their hydrophobic nature; the emulsionant used could: (i) directly influence the adenylate cyclase activity; (ii) induce specific effects on the membrane assembly; (iii) interfere in some way with the odorant effect.

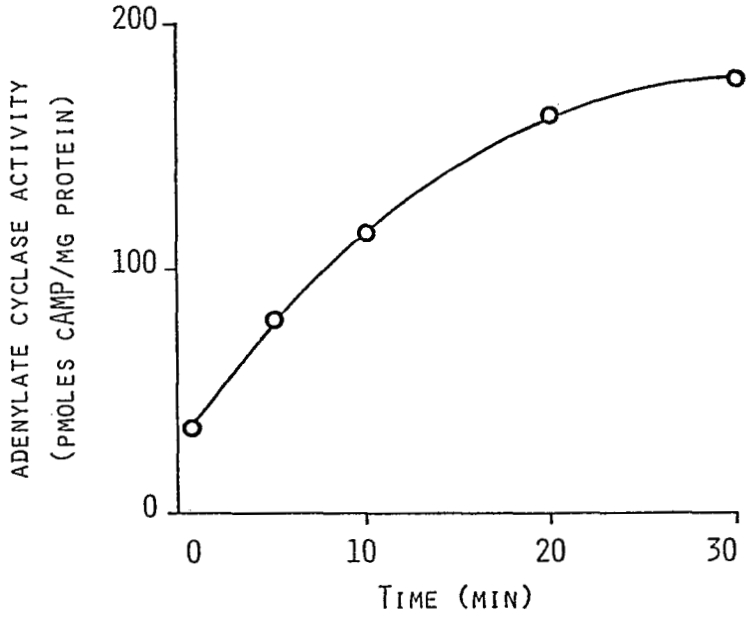

Fig. 4. Time course of adenylate cyclase activity from goat olfactory cilia.

The data here presented indicate that the basic properties of goat olfactory adenylate cyclase are similar not only to those of the olfactory epithelium of other animals (Sklar *et al.*, 1986; Pfeuffer *et al.*, 1989), but also to those of hormone- and neurotransmitter-stimulated enzyme in other tissues (Ferretti *et al.*, 1989; Biondi *et al.*, 1990). Also in goat, as in other animals, the olfactory cilia contain an adenylate cyclase with a specific activity higher than that observed in bulb and olfactory epithelium, mainly in the presence of enzymatic activators. Thus we can speculate that the adenylate cyclase of goat olfactory epithelium is qualified for the transduction of olfactory reception. Aim of future investigations will be the search of the odorant molecules that specifically influence the activity of the enzyme. Since we cannot exclude that the most important second messengers involved in goat olfactory

reception could be IP3 and DAG, we will also test the odorant effects on the synthesis of these latter substance.

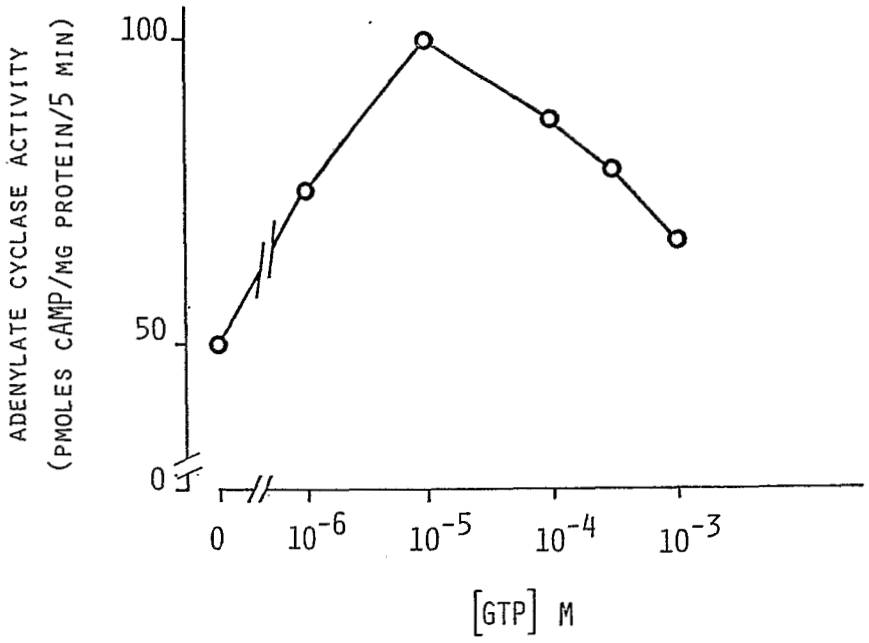

Fig. 5. Modulation of adenylate cyclase activity from goat cilia by increasing GTP concentrations.

Table 1. Effect of 10 μM GTPγS and 10 mM fluoride ions on adenylate cyclase activity from goat olfactory bulb and epithelia. In parenthesis basal values as moles cAMP mg protein^{-1} 5 min^{-1} are reported. Data are the mean of three experiments run in duplicate

	Adenylate cyclase activity (% of basal value)		
	Basal	GTPγS	F
Bulb	100 (63.7 7.0)	266	215
Whole epithelium	100 (50.0 5.5)	159	296
Deciliated epithelium	100 (36.1 4.2)	135	229

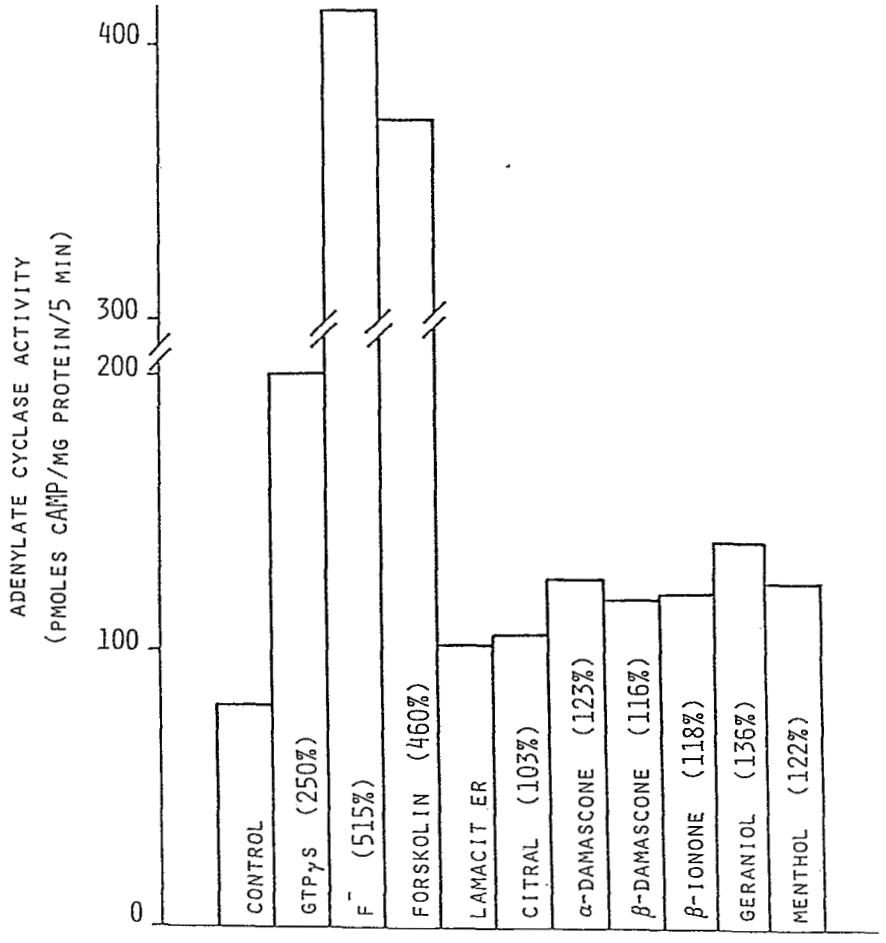

Fig. 6. Effect of adenylate cyclase activators and some odorants on enzyme activity from goat olfactory cilia, GTPγS was 10 μM, F^- mM, odorants 10 μM.

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