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Islet-activating protein discriminates between different inhibitors of thyroidal cyclic AMP system

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TSH-induced cyclic AMP accumulation in dog thyroid slices is inhibited by norepinephrine through an α_2-adrenergic receptor, by carbamylcholine through a muscarinic cholinergic receptor, and by iodide. The inhibitory effect of iodide bears on the adenylate cyclase, but the exact mechanism of its action is still unknown. It is known that norepinephrine acts through activation of the N_i subunit of the cyclase, and that carbamylcholine, activating a phosphodiesterase, acts independently of N_i. IAP (islet-activating protein) has been shown to inactivate the N_i subunit. We studied the effect of IAP on the inhibitory action of iodide, norepinephrine, and carbamylcholine on cyclic AMP accumulation in TSH-stimulated thyroid slices. Incubations of 15 or 22 h, and relatively high concentrations of IAP (250 ng/ml) were necessary to demonstrate an effect of IAP on thyroid slices. We report here that, under those conditions, inhibition of cyclic AMP accumulation by norepinephrine, but not by carbamylcholine or iodide, was suppressed by IAP treatment. These results indicate that the cyclase inhibition by iodide, is either not mediated by N_i, or if mediated by N_i, involves a mode of regulation of this coupling protein that is different from that by which the other 'N_i-mediated' inhibitory hormones act on the enzyme.

IAP *Thyroid* *Cyclic AMP* *Norepinephrine* *Iodide*

1. INTRODUCTION

Thyroid intracellular cyclic AMP concentration in dog stimulated tissue, is negatively regulated by agents acting at the level of its synthesis (norepinephrine, iodide), or its degradation (carbamylcholine). As previously shown [1,2], norepinephrine inhibits the thyroid adenylate cyclase through activation of an α_2-adrenergic receptor. By analogy with other systems [3,4], it has been suggested that norepinephrine inhibits thyroid adenylate cyclase by activating N_i, the guanine nucleotide regulatory subunit of the adenylate cyclase complex, which is involved in hormone-induced inhibition of the enzyme [5–9].

Abbreviations: N_i, inhibitory nucleotide binding regulatory component of adenylate cyclase; TSH, thyroid-stimulating hormone; IAP, islet-activating protein

On the contrary, it has been shown that, in the same system, carbamylcholine does not act at the level of the cyclase, but induces a decrease in cyclic AMP concentration by activating a Ca^{2+}-calmodulin-sensitive phosphodiesterase [10], i.e., in a N_i-independent manner. Finally, iodide, in an organified form, inhibits thyroid adenylate cyclase through a mechanism that is still unknown [11].

Islet-activating protein (IAP), a toxin isolated from the culture medium of *Bordetella pertussis*, activates the adenylate cyclase system of a variety of cell types [12,13]. IAP catalyzes the ADP-ribosylation of a membranous protein which has been identified as the N_i subunit, and thereby blocks the receptor-mediated inhibition of cyclase [14,15]. IAP impairs or abolishes the inhibitory effect of α-adrenergic agonists, dopamine agonists, cholinergic muscarinic or opiate agonists, in platelet membranes, pituitary cells, and neuroblastoma × glioma hybrid cells respectively

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[16–18]. It is thus a useful probe to demonstrate the role of N_i in the action of inhibitory agents on adenylate cyclase. Here, IAP was therefore used for further investigation of the inhibition of thyroid cyclic AMP system by norepinephrine, carbamylcholine, and iodide.

2. MATERIALS AND METHODS

We are very grateful to Dr K.H. Jakobs and Dr G. Schultz for giving us some IAP. TSH was from Armour Pharmaceuticals, Ro 20-1724 from Roche, iodide from Merck, carbamylcholine from ICN Pharmaceuticals, norepinephrine from Sigma, and l-propranolol from ICI.

Thyroids from freshly killed animals were sliced at room temperature, and incubated at 37°C under an atmosphere of O_2:CO_2 (95:5, v/v), in Krebs-Ringer bicarbonate buffer enriched with 8 mM glucose, 0.5 g/l bovine serum albumin, 100 U/ml penicillin, and 100 μg/ml streptomycin. About 40 mg of slices were incubated in 2 ml of medium. IAP was added at the beginning of the incubation, which lasted several hours before addition of iodide, norepinephrine, carbamylcholine, and TSH. Ro 20-1724 (a phosphodiesterase inhibitor), and l-propranolol (a β-antagonist, added in order to block the β-effect of norepinephrine), were added simultaneously with TSH. The incubation was ended by dropping the slices into boiling water for 5 min. The slices were then homogenized, centrifuged, and lyophilized. The tissue extract was resuspended in water, and the cyclic AMP concentration was determined by the method of Gilman, as previously described [19].

3. RESULTS AND DISCUSSION

Table 1 shows that cyclic AMP accumulation in TSH-stimulated dog thyroid slices incubated during 6½ h with IAP, was inhibited by norepinephrine as in control slices. This inhibition was completely suppressed when the incubation with IAP lasted 15 or 22 h. This is consistent with the results obtained by other groups, showing that several hours are necessary to establish IAP effect [20,21]. It must be noted that, after a 24-h incubation, thyroid slices still respond normally to positive and negative control of adenylate cyclase,

Table 1

Time dependency of reversal of norepinephrine inhibitory effect by IAP

	Incubation time		
	6½ h	15 h	22 h
TSH	1155 ± 72	1794 ± 217	1381 ± 142
TSH + norepinephrine	683 ± 30	1042 ± 23	836 ± 69
% inhibition	41	42	39
TSH + IAP	1439 ± 238	2435 ± 14	1900 ± 624
TSH + IAP + norepinephrine	740 ± 81	2423 ± 229	2211 ± 235
% inhibition	49	0	0

IAP was added from the onset of the incubation; norepinephrine, TSH, Ro 20-1724, and l-propranolol were always added 1 h before the end of the incubation. Norepinephrine, Ro 20-1724, and l-propranolol were 10^{-4} M. TSH was 0.5 mU/ml and IAP 2.5 μg/ml in the 6½ h incubation. TSH was 1 mU/ml and IAP 250 ng/ml in the 15 and 22 h incubations. Results are expressed as pmoles cyclic AMP/100 mg wet wt tissue, means of triplicates ± SE

which demonstrates, as shown earlier for other parameters [22], a good survival.

The reversal by IAP of norepinephrine-induced inhibition of adenylate cyclase activity in dog thyroid slices was concentration-dependent. Thyroid slices were incubated 15 h with IAP. TSH 1 mU/ml, 10^{-4} M norepinephrine, 10^{-4} M Ro 20-1724, and 10^{-4} M l-propranolol were added 1 h before the end of the incubation. The inhibition of TSH-induced cyclic AMP accumulation by norepinephrine obtained in the absence of IAP was about 50%. The concentrations of IAP tested were 10, 25, 100, and 250 ng/ml. IAP was only effective at 25 ng/ml, and relieved half of the inhibition at 100 ng/ml. At 250 ng/ml IAP, the norepinephrine inhibition was suppressed.

We studied the effect of IAP treatment on the action of three well known inhibitors of cyclic AMP accumulation in TSH-stimulated thyroid slices: norepinephrine, carbamylcholine, and iodide [2,10,11]. Data presented in table 2, show that only the norepinephrine inhibitory effect was impaired by IAP treatment. As expected, car-

Table 2

Effect of IAP on norepinephrine, carbamylcholine and iodide inhibitory action

	Experiment no.1	% inh.	Experiment no.2	% inh.	Experiment no.3	% inh.
TSH	1679 ± 167		1381 ± 142		1637 ± 25	
TSH + norepinephrine	885 ± 61	47	836 ± 69	39	820 ± 114	50
TSH + iodide	1068 ± 207	36	906 ± 91	34	1337 ± 136	18
TSH + norepinephrine + iodide					877 ± 28	46
TSH + carbamylcholine					1021 ± 16	38
TSH + IAP	2124 ± 432		1900 ± 624		2552 ± 473	
TSH + IAP + norepinephrine	2103 ± 174	0	2211 ± 235	0	2327 ± 401	9
TSH + IAP + iodide	1306 ± 19	39	971 ± 211	49	2193 ± 482	14
TSH + IAP + norepinephrine + iodide					1955 ± 138	23
TSH + IAP + carbamylcholine					1302 ± 258	49

Dog thyroid slices were incubated 22 h, with or without IAP (250 ng/ml). Iodide, 10^{-4} M, was added 14 h after the onset of the incubation. Norepinephrine, l-propranolol, and Ro 20-1724, each 10^{-4} M, carbamylcholine, 10^{-5} M, and TSH, 1 mU/ml, were added 1 h before the end of the incubation. Results are expressed as pmol cyclic AMP/100 mg wet wt tissue ± SE

bamylcholine inhibitory effect, that has been shown to be mediated through an activation by Ca^{2+} of a calmodulin-sensitive phosphodiesterase [10], was not relieved by IAP. The iodide inhibitory effect, although bearing on the cyclase [11], was not relieved by IAP treatment.

One explanation for this result is that iodide could interfere with IAP in such a manner that IAP is no more effective in inactivating N_i. To test this hypothesis, norepinephrine and iodide were both added to IAP-treated and control slices, to see whether or not the presence of iodide prevented the suppression by IAP of norepinephrine inhibitory effect. When norepinephrine and iodide were added to the same TSH-stimulated slices, their inhibitory effects were not additive (table 2). IAP relieved the inhibition of iodide and norepinephrine to the level reached with iodide alone. Moreover, iodide did not interfere with the enhancement of TSH action by IAP. These results indicate that IAP impairs norepinephrine but not iodide effect, and also, that iodide does not hinder IAP effect on norepinephrine inhibitory action.

We have investigated whether or not the absence of effect of IAP on the iodide inhibitory action was specific to the dog thyroid. Using the same experimental conditions, the iodide inhibitory effect on horse thyroid adenylate cyclase was not suppressed in IAP-treated horse thyroid slices (not shown).

In conclusion, IAP, a known inhibitor of the negative subunit N_i of the adenylate cyclase complex [14,15], relieves α_2-adrenergic inhibitory action on dog thyroid adenylate cyclase, but does not impair carbamylcholine inhibitory effect on cyclic AMP accumulation. Thus, the use of IAP allowed us to confirm the results previously obtained in this system [2,10,11], i.e., norepinephrine, but not carbamylcholine, acts through N_i. As the inhibitory effect of iodide was not relieved by IAP, IAP permitted discrimination between two agents, both inhibiting the synthesis of cyclic AMP: norepinephrine, and iodide. The data presented suggest that the inhibition by iodide is not mediated by N_i, or if mediated by N_i, involves a mode of regulation of this coupling protein that is different from that by which the other N_i-mediated inhibitory hormones act on the cyclase. Another example of direct negative regulation on cyclase which may not involve N_i is the recently reported action of progesterone on oocytes [23].

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