ADENOSINE 3',5'-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE AND AMYLASE SECRETION FROM RAT PAROTID GLAND

Takao Kanamori, Taro Hayakawa
and Toshiharu Nagatsu

Department of Biochemistry, School of Dentistry
Aichi-Gakuin University, 2-11 Suemori-dori
Chikusa-ku, Nagoya 464, Japan

Received February 4,1974

SUMMARY

Tolbutamide at a concentration of 10 mM inhibited cyclic AMP-dependent protein kinase in cell-free preparations of rat parotid glands as reported in rat adipose tissues. Incubation of rat parotid slices with 10 mM tolbutamide markedly interfered with the isoproterenol stimulation of amylase secretion. A carboxy derivative of tolbutamide, 1-butyl-3-p-carboxyphenylsulfonylurea, had minimal inhibitory effects both on protein kinase activity and on amylase secretion. These evidences strongly suggest the participation of cyclic AMP-dependent protein kinase in amylase secretion.

Isoproterenol was reported to stimulate markedly amylase secretion from rat parotid gland (1), and the participation of cyclic AMP in isoproterenol-stimulated amylase secretion was also demonstrated (2-4). Futhermore, in rat parotid gland, the existence of cyclic AMP-dependent protein kinase was reported (5). However, it remains to be known whether or not this cyclic AMP-dependent protein kinase participates in amylase secretion. On the other hand, in adipose tissues, Wray and Harris reported the inhibition of cyclic AMP-dependent protein kinase by tolbutamide (6) (Fig.1). This communication deals with inhibition of tolbutamide on the cyclic AMP-dependent protein kinase in cell-free preparations of rat parotid glands and also with interference of the isoproterenol-stimulated secretion of amylase from rat parotid slices.

MATERIALS AND METHODS

Tolbutamide was kindly supplied by Chugai Pharmaceutical Co.,LTD.,Tokyo, and its carboxy derivative,1-butyl-3-p-carboxyphenylsulfonylurea, was kindly

supplied by Yamanouchi Pharmaceutical Co., LTD., Tokyo. Blue starch polymer (Amylase Test "Daiichi") produced by Pharmacia AB, Uppsala was obtained through Daiichi Pharmaceutical Co., LTD., Tokyo.

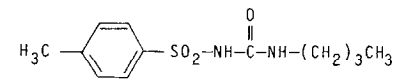
Four fed Wistar rats (150-200 g) were anaesthetized with ether and bled by heart puncture. The parotid glands were removed and immediately placed in a solution of Krebs-Ringer bicarbonate (KRB) medium at 37°C. The KRB medium contained the following supplements (7): 5 mM β-hydroxybutyrate, 10 mM nicotinamide, 10 mM inosine and 0.5 mM adenosine. In all experiments, the medium was gassed by bubbling with a mixture of 5 % CO_2-95 % O_2.

The collected glands were cut with a razor blade into pieces of approximately 1 mm^3, and the slices were washed in 50 ml KRB medium for 1 hour at 37°C. After washing, the slices were divided into 12 sintillation vials and preincubated in 4 ml of KRB or KRB containing 10 mM tolbutamide or its carboxy derivative for 30 min at 37°C. At the end of the preincubation, 10 µl aliquots of the medium were taken to determine amylase activity secreted during preincubation. Isoproterenol (20 µM) was then added at zero time and additionally at 20 min and 40 min during 1 hour-incubation. At the end of the experiment the medium was separated from the slices by filtration. Amylase activities in the medium and the homogenate prepared from the slices were determined by the method using blue starch polymer as substrate (8).

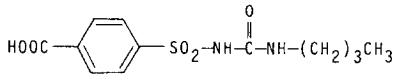
Rat parotid glands were homogenized in 2 volumes of ice-cold 0.25 M sucrose. The homogenate was centrifuged at 100,000 xg for 90 min and a resultant supernatant was used to see the effect of tolbutamide or its carboxy derivative for cyclic AMP-dependent protein kinase. Protein kinase activity was assayed by the method of Reimann et al.(9).

RESULTS AND DISCUSSION

The effect of tolbutamide at various concentrations on cyclic AMP-dependent protein kinase was tested (Fig. 2). Tolbutamide at a concentration of 10 mM inhibited the cyclic AMP-dependent protein kinase activity. Similar inhibition has been observed on the cyclic AMP-dependent protein kinases in



Tolbutamide



1-Butyl-3-p-carboxy-phenylsulfonylurea

Fig. 1. Structures of tolbutamide and its carboxy derivative.

TABLE I

Effects of tolbutamide and its carboxy derivative on isoproterenol-stimulated amylase secretion

Experiment	Isoproterenol-stimulation	Additions		Amylase secreted % of total[a]
Exp. I	−	none		12.5 ± 1.1
	+	none		38.1 ± 1.1
	+	Tolbutamide	10 mM	22.8 ± 0.9
	+	1-Butyl-3-p-carboxyphenyl-sulfonylurea	10 mM	34.7 ± 2.9
Exp. II	−	none		11.3 ± 0.9
	+	none		46.4 ± 4.0
	+	Tolbutamide	10 mM	13.9 ± 1.3
	+	1-Butyl-3-p-carboxyphenyl-sulfonylurea	10 mM	39.7 ± 1.5

[a] The activity of amylase secreted during incubation is expressed as % of total activity of amylase in rat parotid slices at zero time of incubation. All values are mean ± S.E. of triplicated experiments.

adipose tissue (6) and bovine parotid gland (10). A carboxy derivative of tolbutamide, 1-butyl-3-p-carboxyphenylsulfonylurea which has very similar structure to tolbutamide (Fig. 1), had minimal effect on protein kinase activity.

If it is assumed that cyclic AMP-dependent protein kinase has some role as a mediator on the amylase secretion from parotid gland, there will be seen the interference of amylase secretion from rat parotid slices by the presence of tolbutamide. Therefore, the effects of tolbutamide and its carboxy derivative on the isoproterenol-stimulated secretion of amylase were examined using the slice system of rat parotid gland. As shown in Table I, tolbutamide markedly interfered with the amylase secretion. On the contrary, its carboxy derivative, which had a weak inhibitory effect on the protein kinase activity (Fig. 2), had also minimal effect on the amylase secretion. During the prein-

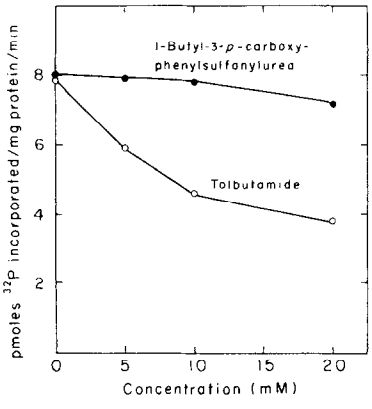


Fig. 2. Effects of tolbutamide and its carboxy derivative on cyclic AMP-dependent protein kinase activity. Each point shows the cyclic AMP-dependent value by subtracting the basal value run without cyclic AMP from the cyclic AMP-stimulated value.

cubation these two drugs had almost no effect on the amylase secretion. These results indicate a good correlation between the inhibitory effect on protein kinase activity and the interfering effect on amylase secretion by tolbutamide and its carboxy derivative.

Though it still remains as a questionable point whether or not tolbutamide specifically inhibits cyclic AMP-dependent protein kinase in rat parotid slice system, these two evidences on the inhibitory effects of tolbutamide

strongly suggest the possibility of the participation of the cyclic AMP-dependent protein kinase in amylase secretion from rat parotid gland.

This is the first report of tolbutamide action on the amylase secretion from parotid gland where its effect can be explained by its inhibition on cyclic AMP-dependent protein kinase. An experiment is now going on to confirm the participation of cyclic AMP-dependent protein kinase on amylase secretion by trying to find out an intrinsic substrate for the protein kinase which may relate to the mechanism of amylase secretion.

ACKNOWLEDGEMENT

The authers would like to thank Drs. A. Niki and H. Niki for their helpful suggestions during the course of this work. The authers also thank Dr. K. Kato of Nagoya University for his kind supply of ^{32}P-ATP.

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