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SHORT COMMUNICATIONS

Mechanism of the pilocarpine-stimulated increase in rat submaxillary gland amylase level

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SCHNEYER and Schneyer[1] have shown that the amylase level in the submaxillary gland of the starved rat may be increased 20-fold either by feeding or by injection with acetylcholine or pilocarpine. This phenomenon, which may be observed after only 20 min of stimulation, can be abolished by pretreatment with atropine.[1] Three possible mechanisms were proposed to account for the observed increase in amylase level: (1) activation of a proenzyme (2) increased serum space in the gland or an increase in serum amylase levels, or (3) synthesis of new enzyme by the submaxillary gland tissue cells. Since attempts to explain the increase in terms of the first two mechanisms failed, it was assumed that submaxillary gland amylase was being synthesized *de novo* under the control of the parasympathetic system.[1] However, the experiments reported here show that the incorporation of ^{14}C-labeled amino acids into rat submaxillary gland amylase decreased after the administration of pilocarpine. Furthermore, both actinomycin D and puromycin (potent inhibitors of *de novo* protein synthesis[2, 3]) failed to block the pilocarpine-stimulated increase in rat submaxillary gland amylase levels. These results suggest that pilocarpine does not stimulate the *de novo* synthesis of rat submaxillary gland amylase.

METHODS

Male Long–Evans rats (200 g) were starved for 48 hr before the start of each experiment. Actinomycin D was dissolved in saline and injected i.p. Puromycin hydrochloride was dissolved in saline and given in an initial dose of 12 mg, followed by 6 mg every hour during the experiment. Injection (s.c.) of pilocarpine hydrochloride (2 mg) was started 1 hr after the initial dose of either actinomycin D or puromycin and repeated every half hour. Four hr after the commencement of the pilocarpine injections, the rats were sacrificed and their submaxillary glands were removed, weighed and homogenized (Duall tissue grinder) in 10 vol. of ice-cold 0·067 M phosphate buffer (pH 6·8) containing 0·014 M sodium chloride. After the homogenate had been centrifuged at 500 *g* for 5 min, the supernatant solution was assayed for amylase activity by the method of Bernfeld.[4] Results are expressed as mg of maltose released/mg of wet tissue in 15 min at 37°. The results in Tables 2 and 3 (Exp. 1) were subjected to an analysis of variance followed by a studentized range test at the $P = 0{\cdot}05$ level.[5]

The incorporation of ^{14}C-labeled amino acids into submaxillary gland amylase and total protein was studied in starved rats (48 hr) which had received pilocarpine (2 mg, s.c.) every 30 min for 3½ hr. Each animal was then injected with 4 μc of a mixture of uniformly labeled ^{14}C amino acids (New England Nuclear) and sacrificed 20 min later. Submaxillary gland amylase was isolated and purified by the glycogen method of Loyter and Schramm,[6] as modified by Gromet-Elhanan and Winnick.[7] This procedure results in an enzyme of high purity; further attempts at purification did not result in an increase in enzyme activity. The relatively low amylase activity found in the rat submaxillary gland[1] made it necessary to combine the glands from each group of animals in order to increase the recovery of enzyme. Total proteins were isolated by the method of Dawkins *et al.*[8] Aliquots of the amylase and total protein were dissolved in hyamine and counted in a liquid scintillation spectrometer. Protein was determined by the method of Lowry *et al.*[9]

RESULTS AND DISCUSSION

Rat submaxillary gland amylase levels rose slowly during the first 2 hr of pilocarpine stimulation, then increased sharply to reach a maximum after 4–5 hr.[1] Injections of ^{14}C-labeled amino acids were made 3½ hr after the commencement of pilocarpine stimulation, when the rate of increase in amylase

levels was greatest. Results from a typical experiment will be found in Table 1. The level of amylase in glands from pilocarpine-treated animals was seven times that found in the controls. Nevertheless, amylase isolated from the submaxillary glands of rats receiving pilocarpine contained 45 per cent

TABLE 1. EFFECT OF PILOCARPINE ON THE INCORPORATION OF ^{14}C-LABELED AMINO ACIDS INTO PURIFIED AMYLASE AND TOTAL PROTEINS FROM RAT SUBMAXILLARY GLAND

Treatment	Amylase activity*		Radioactivity (cpm/mg protein)	
	Homogenate†	Purified amylase‡	Total gland protein‡	Purified amylase‡
Control	1·58 ± 0·28	3340	176	160
Pilocarpine	11·20 ± 2·0	3600	152	90

* Results for the whole homogenate are expressed as mg maltose/mg total gland protein/15 min. Results for the purified enzyme are expressed as mg maltose/mg amylase protein/15 min. Protein was determined by the Lowry[9] method with bovine serum albumin as a standard.

† Each result is the mean from 5 animals ± S.E.

‡ These results were obtained with pooled homogenates from 5 animals.

fewer counts than amylase from control animals. In a similar experiment using ^{14}C-labeled leucine, a 37 per cent reduction in amylase labeling was observed in pilocarpine-treated animals.

Puromycin hydrochloride had no significant ($P > 0·05$) effect on the pilocarpine-stimulated increase in submaxillary gland amylase (Table 2). Similar results (Table 3, Exp. 1) were obtained with actinomycin D (0·5 mg/kg). However, when the dose of actinomycin D was increased to a near-lethal level

TABLE 2. EFFECT OF PUROMYCIN HYDROCHLORIDE ON THE INCREASE IN RAT SUBMAXILLARY GLAND AMYLASE PRODUCED BY PILOCARPINE INJECTION

Treatment	Amylase activity* (mg maltose/mg wet tissue/15 min)
Control	0·11 ± 0·018
Pilocarpine	2·92 ± 0·72
Pilocarpine + puromycin	4·19 ± 1·06
Puromycin	0·19 ± 0·015

* Each result is the mean from 5 animals ± S.E.

(2·5 mg/kg), significant ($P < 0·001$) potentiation of the pilocarpine effect was observed (Table 3, Exp. 2). Both puromycin and actinomycin D are potent inhibitors of *de novo* protein synthesis in a wide variety of systems.[2, 3] In addition, McGeachin *et al.*[10] found that the rate of amylase synthesis in the isolated perfused liver was greatly decreased by puromycin. Nevertheless, it was not possible to inhibit the pilocarpine-stimulated increase in amylase level by treatment with either of these antibiotics. The potentiation of the pilocarpine effect at the high dose level (2·5 mg/kg) of actinomycin D (Table 3, Exp. 2) is probably due to an inhibition of the *de novo* synthesis of catabolic enzymes. Similar results have been reported for several adaptive enzymes in the rat liver.[11] The potentiation of the pilocarpine effect by puromycin (Table 2) is not statistically ($P > 0·05$) significant.

The data obtained with puromycin and actinomycin D (Tables 2 and 3) are not consistent with the hypothesis that the pilocarpine-stimulated rise in submaxillary gland amylase levels results from an increase in the *de novo* synthesis of the enzyme.[1] This conclusion is supported by the experiments in

which the incorporation of ^{14}C-labeled amino acids into amylase was studied (Table 1). The decrease in the specific activity of amylase isolated from pilocarpine-treated rats could represent dilution by preformed nonradioactive enzyme.[1] However, in no experiment was the decrease large enough to account for the dramatic increase in amylase levels observed during pilocarpine stimulation. The decrease could also result from an increase in the submaxillary gland free amino acid pool,[12] although the total gland proteins consistently showed a smaller change in a specific activity (Table 1). It is well known that both synthesis and degradation play important roles in controlling the level of tissue enzymes.[13] Thus if, under the influence of pilocarpine, the rate of amylase catabolism decreased while

TABLE 3. EFFECT OF ACTINOMYCIN D ON THE INCREASE IN RAT SUBMAXILLARY GLAND AMYLASE PRODUDED BY PILOCARPINE INJECTION

Treatment	Amylase activity* (mg maltose/mg wet tissue/15 min)
Experiment 1	
Control	0·059 ± 0·004
Pilocarpine	1·68 ± 0·30
Pilocarpine + actinomycin D†	2·07 ± 0·14
Actinomycin D†	0·077 ± 0·013
Experiment 2	
Pilocarpine	1·66 ± 0·15
Pilocarpine + actinomycin D‡	4·54 ± 0·17

* Each result is the mean from 5 animals ± S.E.
† 0·5 mg/kg.
‡ 2·5 mg/kg.

the rate of synthesis remained the same, then the enzyme level would rise. Such a situation is consistent with the results presented in this paper.

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