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Enlargement of salivary glands in rats after chronic administration of physalaemin or isoprenaline

G. BERTACCINI, G. DE CARO AND R. CHELI

Chronic administration of physalaemin and isoprenaline to rats produced a significant salivary glands enlargement which was moderate (34% fresh weight increase and 48% dry weight increase compared with control glands) after the peptide administration and striking after the amine (630% fresh weight increase and 600% dry weight increase compared with control glands). Physalaemin caused only hypertrophy of the gland, whereas isoprenaline induced a marked hyperplasia.

PHYSALAEMIN, a polypeptide recently found in the skin of the South American amphibian *Physalaemus fuscumaculatus*, (Erspamer, Anastasi, Bertaccini & Cei, 1964; Bertaccini, Cei & Erspamer, 1965) was shown to possess a powerful sialagogic activity particularly evident in dogs and rats (Bertaccini & De Caro, 1965). In these animals the threshold salivatory dose by intravenous injection was less than 1 μg/kg and its effect exceeded by 7 to 250,000 times that of the most common sialogenous drugs.

Physalaemin had no detectable effect on the autonomic nervous system but seemed to exert a direct effect on the salivary glands.

Since chronic administration of isoprenaline to rats is followed by hypertrophy of salivary glands (Selye, Veilleux & Cantin, 1961; Wells, 1962; Schneyer, 1962; Pohto & Paasonen, 1964) physalaemin was administered chronically to rats to find out if it had a similar effect and if so, what were the histological changes.

Experimental

MATERIALS AND METHODS

Young female albino rats of the Wistar strain (Morini Farm, S. Polo) weighing 110 $\pm$ 2·4 g were used. The diet consisted of common laboratory chow with water *ad libitum*. Two groups of 12 rats each, received 10 μg/kg and 100 μg/kg respectively of physalaemin, four times daily, for 12 days. Two groups of 15 rats each, received isoprenaline, 400 mg/kg once daily and 50 mg/kg twice daily respectively, for 12 days. The peptide and the amine were dissolved in 0·5 ml of distilled water and injected intraperitoneally. One group of 20 rats received only distilled water intraperitoneally. Animals were weighed every other day. Control and treated animals were killed 24 hr after the last injection. At autopsy the parotid, submaxillary and major sublingual salivary glands were dissected free of extraneous tissues and weighed together. The dry weight of the glands was determined after drying the glands in an oven for 48 hr at about 100°. From each group of rats some salivary glands

From the Institute of Pharmacology and the Veterinary Surgical Clinic, University of Parma, Parma, Italy.

were fixed in 10% formalin solution for subsequent staining with haematoxylin and eosin. Brain, lungs, liver, spleen and kidneys were also removed, weighed and examined histologically.

Results

All the animals increased in weight (Table 1). Rats treated with physalaemin showed a remarkable flushing of the ears, snout and paws which appeared immediately after the injection; in addition, increased salivation and occasionally nasal and lachrymal secretion were noted with the larger dose (100 μg/kg). Full recovery took 15 to 30 min.

TABLE 1. INCREASE IN TOTAL BODY WEIGHT FOLLOWING ADMINISTRATION OF PHYSALAEMIN OR ISOPRENALINE TO RATS

Treatment			Body weight		
Drug	Dose	No. of rats	Initial, g ± s.e.	Final, g ± s.e.	% Increase
None	—	20	112·5 ± 1	150 ± 2·7	34
Physalaemin ..	10 µg/kg 4 times daily	12	111 ± 1·1	148 ± 3·8	33·5
Physalaemin ..	100 µg/kg 4 times daily	12	110 ± 1·3	143 ± 3	30
Isoprenaline ..	50 mg/kg twice daily	15	111·5 ± 1	148 ± 3	32·5
Isoprenaline ..	400 mg/kg once daily	15	113·5 ± 1·5	125 ± 4·5	14

Rats treated with isoprenaline showed a profuse salivation which became evident within 5 to 10 min after the injection; saliva flowed out of the mouth moistening the fur around the lower lip and mandible. This was particularly evident in the rats treated with the higher dose of the amine (400 mg/kg) and especially during the first two or three days of treatment. In this period most of the body was moistened.

About 20 min after injection the rats lay prostrate on the floor of the cage and a generalized piloerection was also noticed. Within 3 to 6 hr however, they appeared to have recovered except for a marked thirst which lasted all day.

After a decrease in weight during the first 3 or 4 days of treatment, the general condition of the rats improved although the final increase in weight was only 14% (control rats increase = 34%).

The effects of these drugs on the fresh and dry weights of salivary glands are shown in Table 2. Physalaemin caused an increase in the wet and dry

TABLE 2. SALIVARY GLANDS WEIGHTS OF RATS AFTER TREATMENT WITH PHYSALAEMIN OR ISOPRENALINE

Treatment			Salivary glands weight in mg/kg	
Drug	Dose	No. of glands	Fresh, mg ± s.e.	Dry, mg ± s.e.
None	—	15	399 ± 13	112 ± 5
Physalaemin ..	10 µg/kg	8	535 ± 34	166 ± 16
Physalaemin ..	100 µg/kg	8	415 ± 22	114 ± 9·5
Isoprenaline ..	50 mg/kg	10	2537 ± 141	688 ± 40
Isoprenaline ..	400 mg/kg	10	2012 ± 93	538 ± 25

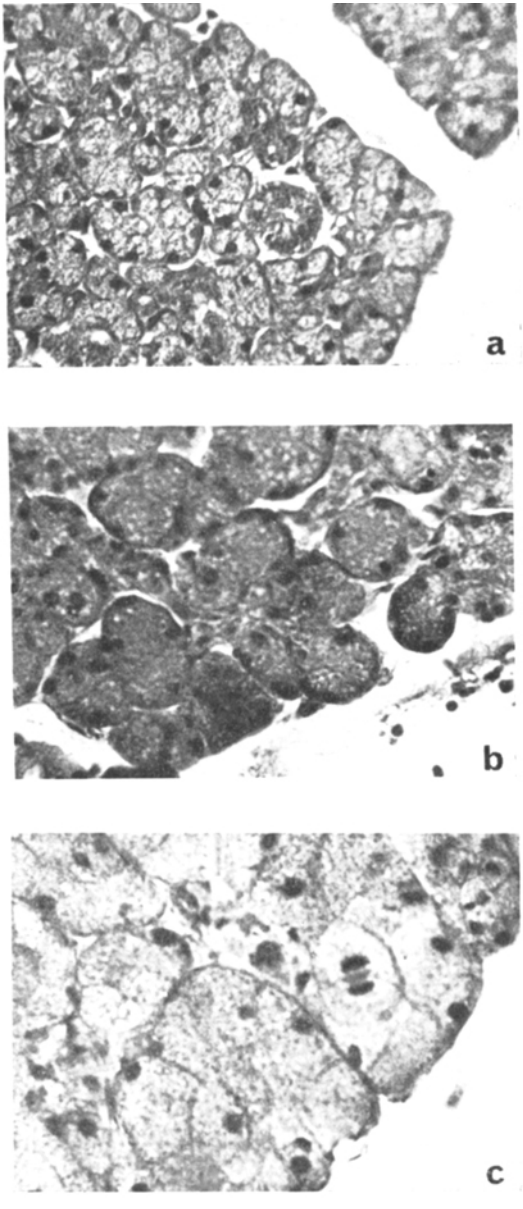

FIG. 1. Histological pattern of parotid glands from control rats (a), rats treated with physalaemin (b) and rats treated with isoprenaline (c).

weights of salivary glands which was statistically significant ($P < 0.02$) with the low dose of the peptide but very slight (4%) and non-significant ($P > 0.4$) with the dose of 100 μg/kg. Isoprenaline caused the usual striking enlargement of the glands already observed by many authors. The increase in weight of the gland was less pronounced, though evident, in the rats receiving the higher dose of the amine.

Histological examination showed differences between glands from rats treated with physalaemin and those from rats treated with isoprenaline. With physalaemin treatment hypertrophy was the major change; increase in cell size was moderate but enough to account for the increase observed in gland weight. Areas of necrosis were never seen nor were mitotic aspects visible (Fig. 1b). With isoprenaline there was not only an extraordinary increase in cell size but also some hyperplasia (Fig. 1c) as shown by the presence of numerous mitoses and of several nuclei in the phase of spirema.

On gross and histological examination the parenchymatous organs of rats treated with physalaemin were practically normal. The fresh weights did not differ from those obtained from the equivalent tissues of control animals (Table 3).

TABLE 3. FRESH WEIGHT OF DIFFERENT PARENCHYMATOUS ORGANS OF RATS AFTER TREATMENT WITH PHYSALAEMIN OR ISOPRENALINE

Treatment		Fresh weight in g per 100 g rat ± s.e.				
Drug	Dose	Brain	Lungs	Liver	Spleen	Kidneys
None	—	0·94 ± 0·2	0·78 ± 0·03	4·51 ± 0·26	0·36 ± 0·02	0·90 ± 0·04
Physalaemin ..	10 μg/kg	0·99 ± 0·07	0·76 ± 0·05	4·23 ± 0·15	0·54 ± 0·09	0·94 ± 0·05
Physalaemin ..	100 μg/kg	1·07 ± 0·14	0·90 ± 0·09	4·06 ± 0·2	0·49 ± 0·08	1 ± 0·1
Isoprenaline ..	50 mg/kg	0·98 ± 0·03	0·86 ± 0·07	5·53 ± 0·34	0·55 ± 0·13	0·97 ± 0·04
Isoprenaline ..	400 mg/kg	0·85 ± 0·05	0·74 ± 0·02	5·15 ± 0·19	0·45 ± 0·04	0·83 ± 0·03

Discussion

Physalaemin, 10 μg/kg, selectively increased the weights of salivary glands in rats, whereas a higher dose (100 μg/kg) did not.

Isoprenaline was much more effective and again the effect was more striking with the lower dose, probably due to a toxic action in the larger dose (Pohto & Paasonen, 1964). Indeed, in our experiments rats treated with 400 mg/kg of isoprenaline showed little increase in weight compared with controls. With physalaemin it is more difficult to explain the lack of effect of the higher dose of the peptide and in fact the total increase in body weight and the conditions of the organs examined were quite satisfactory.

The dose of isoprenaline (400 mg/kg) was that used by Selye & others (1961) to cause evident enlargement of the glands. Bertaccini & De Caro (1965) found in acute experiments that physalaemin was 100,000 times more potent in its salivatory effects on the rat than isoprenaline, hence the large difference in dosage.

The fact that there was also an increase in dry weight after physalaemin (10 μg/kg), suggested that there was a real increase in non-aqueous

cellular material. Histological examination also showed a moderate cellular hypertrophy. Thus physalaemin as a potent sialagogue could be expected to cause a hypertrophy from hyperfunction. In contrast the hyperplasia observed after isoprenaline is probably due to a powerful and selective stimulation of salivary gland growth (Selye & others, 1961).

The sialogenous action of physalaemin, which is even more striking in the acute experiment (Bertaccini & De Caro, 1965) must be regarded as only a side-effect, when compared with the hypotensive action of the peptide (threshold hypotensive dose 10–30 times lower than threshold salivatory dose in rats and over 1000 times lower in dogs).

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