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THE "DIRECT" EFFECT OF PHYSALAEMIN ON SALIVARY GLAND CELLS

BY

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It is generally assumed that salivary gland cells can be excited only by the transmitters of their secretory nerves or by drugs which mimic the action of the transmitters. Since the time of Claude Bernard (1856) it has been agreed that these cells cannot be excited electrically, and the sialogogue effects even of agents like potassium ions (Feldberg & Guimarais, 1936) or histamine (Dale & Laidlaw, 1910) can be abolished by atropine (see Emmelin, 1967). Recently, however, a "direct" sialogogue effect has been attributed to physalaemin. This endecapeptide, originally extracted from the skin of the South American amphibian *Physalaemus fuscumaculatus* by Erspamer, Bertaccini & Cei (1962) and later synthesized by Bernardi, Bosisio, Goffredo & De Castiglione (1964), was found to have a powerful secretory effect on some salivary glands (Erspamer, Anastasi, Bertaccini & Cei, 1964; Bertaccini, Cei & Erspamer, 1965). This effect was analysed by Bertaccini & De Caro (1965), who found that it was particularly pronounced in dogs and rats and that it could be abolished neither by atropine nor by sympatholytic agents. The action was therefore described as "direct," although it was pointed out that an action *via* adrenergic β-receptors could not be entirely ruled out; the only β-receptor blocking drug available at that time was dichloroisoprenaline, and its blocking action in salivary glands is less marked than its secretory effect. Adrenergic β-receptors are present in salivary glands of rats, but α-receptors dominate (Emmelin, Holmberg & Ohlin, 1965). Recently it was observed that the catecholamine receptors of the submaxillary gland cells of dogs belong exclusively to the β-type (Emmelin & Holmberg, 1967a). In view of the fact that physalaemin was described as particularly active on the dog's submaxillary gland and on salivary glands of rats it was considered desirable to try the effect of modern, effective β-blocking agents on the sialogogue activity of physalaemin.

METHODS

The experiments were made on 6 dogs anaesthetized with chloralose and urethane (50+500 mg/kg intravenously after induction with ether). The submaxillary and parotid ducts were exposed and cannulated. Synthetic physalaemin was injected either through a cannula in a femoral vein or through the submaxillary or parotid ducts. In the latter case the drug, dissolved in 0.3–0.5 ml. physiological saline solution, was injected in retrograde direction as described previously (Emmelin, Muren & Strömblad, 1954). All other drugs were given intravenously. In one dog the auriculo-temporal nerve and the chorda tympani of one side were cut 23 days before the acute experiment. This operation was caried out under pentothal anaesthesia.

RESULTS

Our investigations on the submaxillary gland confirm the observations of the Italian authors. Physalaemin injected intravenously was found to evoke a flow of saliva. This was not abolished by parasympatholytic agents. Atropine (0.4 mg/kg) and αα-diphenyl-γ-piperidinobutyramide (Hoechst 9980, 1 mg/kg) were used. Nor was the secretory effect abolished by drugs with blocking action on α-receptors for catecholamines; as such dihydroergotamine (0.3 mg/kg) and phenoxybenzamine (1 mg/kg) were given.

In addition the following findings were also made. Secretion could be elicited by injecting physalaemin through the salivary duct into the submaxillary gland. A dose of 1 μg was usually given. No general circulatory or respiratory effects were then obtained, as was the case when the polypeptide was administered intravenously. Previous section of the preganglionic parasympathetic secretory fibres of the submaxillary gland rendered the gland cells supersensitive to physalaemin. This can be seen in Fig. 1. This figure

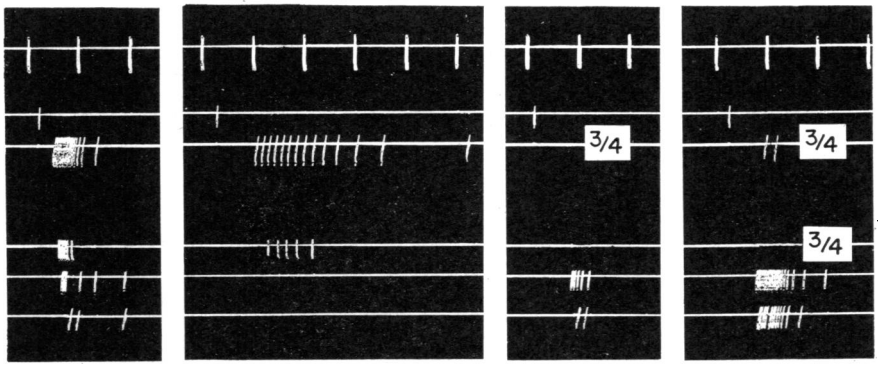

Fig. 1. Dog, 15 kg. Right chorda tympani and auriculotemporal nerves cut 23 days previously. Records from above: min; signal to mark intravenous injections; secretion from right; from left submaxillary gland; from right; from left parotid gland. First injection: 2 μg methacholine/kg; second: 2 μg isoprenaline/kg; third: 1 μg; and fourth: 2 μg physalaemin/kg.

also demonstrates the marked secretory effect of the β-receptor stimulating isoprenaline on the submaxillary gland of the dog. The β-receptor blocking compounds used were propranolol and D-(−)-N-isopropyl-p-nitrophenyl ethanolamine (INPEA). Figure 2 illustrates the action of INPEA. A dose of 5 mg/kg strongly reduced the response to isoprenaline and an additional dose of 5 mg/kg abolished it. The secretory effects of physalaemin and methacholine were not affected. Similar results were obtained with propranolol 1–2 mg/kg.

Physalaemin was also found to cause salivation from the parotid gland and the effect was even more marked than that on the submaxillary gland, as shown in Fig. 1. Secretion was obtained both when the polypeptide was injected intravenously and when given through the salivary duct. The response was still obtained after degeneration of the post-ganglionic parasympathetic secretory fibres contained in the auriculotemporal nerve, and it was in fact found to be augmented (Fig. 1). Propranolol and INPEA did not change the secretory effect of physalaemin; this is not surprising considering the fact

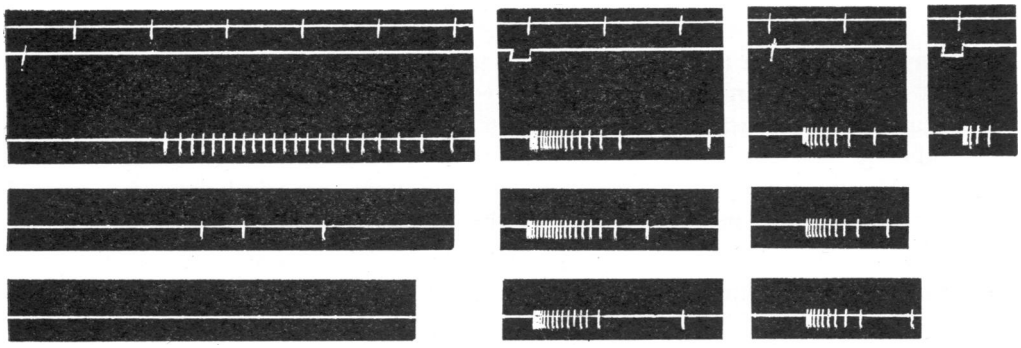

Fig. 2. Dog, 12 kg. Records from above: min; signal to mark injections; secretion from the submaxillary gland before; 10 min after INPEA 5 mg/kg; and 10 min after a further dose of INPEA 5 mg/kg. First injection: isoprenaline, 2 μg/kg intravenously; second injection: physalaemin, 1 μg/kg, in 0.5 ml. saline solution, injected through the salivary duct over a time period of 5 sec. The constriction of the cannula was released 10 sec later. Third injection: methacholine, 1 μg/kg intravenously. Fourth injection: 0.5 ml. saline solution injected through the salivary duct.

that the parotid gland of the dog lacks β-receptors (Emmelin & Holmberg, 1967a; cf. Fig. 1). The response was not abolished by α-receptor blocking agents either, or by parasympatholytics. In the experiment of Fig. 3 Hoechst 9980, 0.1 mg/kg, annulled the secretory effect of methacholine; but ten times the dose of the parasympatholytic drug did not reduce the sialogogue response to physalaemin.

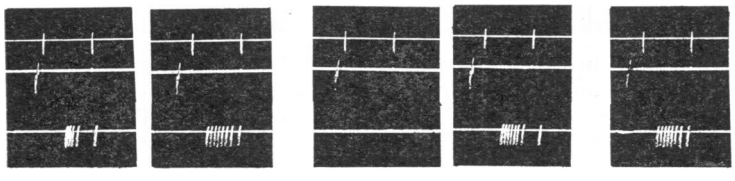

Fig. 3. Dog, 10 kg. Records from above: min, signal to mark intravenous injections; secretion from the parotid gland. First and third injections: methacholine, 2 μg/kg. Second, fourth and fifth injections: physalaemin, 1 μg/kg. Injection of Hoechst 9980, 0.1 mg/kg between sections 2 and 3, and of 1 mg/kg between sections 4 and 5.

DISCUSSION

The conclusion, of the previous investigators, that physalaemin exerts its secretory effect locally in the salivary glands, and not by acting, for example, on the central nervous system or the adrenal medulla, is supported by the present finding that salivation is evoked when the polypeptide is injected through the salivary duct. In the parotid gland secretion was found to occur in response to physalaemin even when the post-ganglionic secretory fibres of the auriculotemporal nerve had degenerated. In the dog this gland seems to lack sympathetic secretory fibres. It is true that some parasympathetic secretory fibres seem to reach the gland by anatomically unknown pathways (see Emmelin & Holmberg, 1967b); but the observation that a very marked secretory response was

obained after previous section of the auriculotemporal nerve, in fact considerably larger than that of the innervated gland, indicates that the secretory activity of physalaemin is not dependent on glandular nerve fibres. In confirmation of the work of Bertaccini & De Caro (1965) it has been found that neither parasympatholytic nor adrenergic α-blocking agents are able to prevent the secretory effect of physalaemin. The possibility of an action *via* β-receptors is excluded by the observations that effective β-blockers do not abolish the response of the submaxillary gland to physalaemin and that flow of saliva is elicited in the parotid gland containing no β-receptors. Physalaemin thus seems to provide an example of drugs causing salivation by a hitherto unknown mode of action on salivary gland cells, differing from that of all so far used sialogogue compounds ; this very likely applies to the related endecapeptide eledoisin as well (Bertaccini & De Caro, 1965). Physalaemin resembles most other sialogogues in the respect that supersensitivity towards it develops after parasympathetic decentralization or denervation.

SUMMARY

1. The endecapeptide physalaemin has a marked secretory effect on the submaxillary gland of the dog, as shown by previous investigators. On the parotid gland its sialogogue effect is even more pronounced. Secretion can be evoked in both glands when the drug is applied locally, through the salivary duct. The secretory response is increased by previous parasympathetic decentralization or denervation.

2. Secretion is obtained not only after administration of parasympatholytic and α-adrenergic blocking drugs, as demonstrated earlier on the submaxillary gland, but also after the β-receptor blocking agents propranolol and INPEA.

3. These findings suggest that physalaemin causes salivation by a mode of action on the gland cells differing from that of other sialogogue agents so far investigated.

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