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Salivation induced by prostaglandin $F_{2\alpha}$ and modification of the response by atropine and physostigmine

R. A. HAHN AND P. N. PATIL

The Ohio State University, College of Pharmacy, Columbus, Ohio 43210, USA

Summary

1. Administration of $PGF_{2\alpha}$ to the anaesthetized dog produced dose-related salivation accompanied by weak pressor and negative cardiac chronotropic effects. Injection of PGE_1 did not produce salivation.

2. Electrical stimulation of the chorda tympani nerve or injection of $PGF_{2\alpha}$ produced salivary responses which were not affected by pretreatment with phentolamine, but were abolished by pretreatment with atropine. Treatment with hexamethonium reduced the response to nerve stimulation but did not alter the response to $PGF_{2\alpha}$.

3. Pretreatment with physostigmine augmented the salivary response to both nerve stimulation and $PGF_{2\alpha}$.

4. These experiments suggest that salivation produced by $PGF_{2\alpha}$ is probably due in part, to liberation of acetylcholine from cholinergic nerve terminals. These results are consistent with previously proposed modulatory functions of prostaglandins on neurotransmission.

Introduction

Prostaglandins, a group of naturally occurring fatty acids, can produce a variety of effects in man and animals (von Euler & Elisson, 1967; Bergström, Carlson & Weeks, 1968; Ramwell & Shaw, 1971).

While conducting experiments on the anaesthetized dog it was noted that salivation occurred after administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Injection of prostaglandin E_1 (PGE_1) did not result in salivation. Pretreatment of animals with atropine abolished the salivary response produced by $PGF_{2\alpha}$. In view of the proposed neurotransmitter modulatory functions of prostaglandins (Hedqvist & Brundin, 1969; Kadovitz, Sweet & Brody, 1971a, 1971b), this effect of $PGF_{2\alpha}$ appeared quite interesting.

Since a search of the literature did not reveal information concerning salivation produced by $PGF_{2\alpha}$, the present study was undertaken to determine the mechanism by which this effect is produced.

Methods

Adult mongrel dogs of either sex were anaesthetized intraperitoneally with pentobarbitone sodium (35 mg/kg). The trachea was intubated and the animals allowed

to respire spontaneously. Arterial blood pressure (1 mmHg≡1·333 mbar) was measured from the right femoral artery using a Statham transducer (P23AC). Lead II electrocardiogram was recorded by means of small subcutaneous needle electrodes. In addition, to obtain instantaneous changes in heart rate, the latter signal was relayed to a cardiotachometer which was triggered by the R wave of the electrocardiogram. Drug solutions were administered through the cannulated right femoral vein.

The duct of the left submaxillary salivary gland (Wharton's duct) was approached through a midline skin incision extending from the symphysis of the jaw to the hyoid. The mylohyoid muscle was then sectioned just lateral to the midline, for a distance of about 8 cm. Wharton's duct was isolated and cannulated with a length of polyethylene tubing (Clay Adams, PE 50). Drops of saliva from the tubing were directed to fall into a small cup, suspended from a force-displacement transducer (Grass, FTO3C). As each drop of saliva hit the cup a deflection (with increased tension) was recorded by one pen of the recorder. Salivary response was arbitrarily expressed as the number of drops of saliva collected within 2 min of the appearance of the first drop of saliva. This period encompassed the entire salivary response to nerve stimulation and most of the salivary response to $PGF_{2\alpha}$. In those rare cases where a baseline salivary flow was observed, the baseline flow occurring within the 2 min immediately preceding either nerve stimulation or $PGF_{2\alpha}$ administration was subtracted from the response obtained to these procedures.

The parasympathetic nerve supply to the left submaxillary salivary gland (chorda tympani) was prepared for electrical stimulation in the following manner. The chorda tympani was identified at the level of the lingual nerve, running caudad across the sublingual salivary gland. The chorda tympani was isolated, a small suture tied firmly around it, and the nerve was sectioned central to the tie. The nerve was then placed on small bipolar electrodes connected to an electronic stimulator (Grass, Model SD9). Finally, the entire area was covered with mineral oil.

All directly measured parameters were recorded on a multichannel direct-writing oscillograph (Grass, Model 7). Fifteen minutes were allowed between the end of the surgical procedure and the commencement of the experiment.

To determine the relationship between salivary response and dose of $PGF_{2\alpha}$ animals were injected with graded doses of the prostaglandin, spaced 10 min apart. The results obtained from this series of experiments were then averaged and plotted semilogarithmically as salivary response (drops/2 min) versus dose of $PGF_{2\alpha}$ (μg/kg).

To determine the effect of various pretreatments on the salivary response to nerve stimulation or $PGF_{2\alpha}$, the following experimental sequence was utilized. After the period of equilibration each animal received two control periods of chorda tympani nerve stimulation (supramaximal voltage, 25 Hz, 10 ms duration, for 15 s), and two control injections of $PGF_{2\alpha}$ (8 μg/kg), spaced at 10 min intervals. Ten minutes elapsed, and the animal was given a drug or saline infusion. After 10 min each animal was rechallenged once with nerve stimulation and once with $PGF_{2\alpha}$ (8 μg/kg), spaced 10 min apart. After another 10 min this sequence was repeated with a second drug or saline infusion. Thus, after obtaining control responses each animal received either two drug or two saline infusions and was challenged after each with nerve stimulation and $PGF_{2\alpha}$. The average of the two control responses to nerve stimulation was taken as 100% response for this procedure in a given animal. Salivary responses to nerve stimulation after drug treatments were then calculated

as a percentage of the control response. Similar calculations were performed in the case of $PGF_{2\alpha}$, expressing salivary responses to $PGF_{2\alpha}$ after drug treatments as a percentage of its control, in each animal. The results from four to five animals receiving the same treatment were then averaged and plotted as salivary response (% of control) versus elapsed time of the experiment (min). Zero time during these experiments was defined to be that time at which the second control salivary response was obtained to either nerve stimulation or $PGF_{2\alpha}$. Salivary responses recorded after the first drug or saline infusion represent an elapsed time of 30 min from the final respective control response, while those recorded after the second drug or saline infusion represent an elapsed time of 60 minutes. Mean responses to nerve stimulation and $PGF_{2\alpha}$ recorded after various drug treatments were compared with mean values obtained in saline-treated animals, for the corresponding procedure. Statistical significance between means was assessed by the Student's *t* test.

The following drugs and doses (expressed as salts) were used: hexamethonium chloride (10 mg/kg), phentolamine methanesulphonate (2 mg/kg), atropine sulphate (1 mg/kg), and physostigmine sulphate (100 μg/kg). The prostaglandins used in this study were kindly supplied by Dr. John Pike, The Upjohn Company. Stock solutions of prostaglandins were made in 95% ethanol and kept in the refrigerator. Appropriate dilutions were made fresh in saline on the day of the experiment.

Results

The intravenous injection of $PGF_{2\alpha}$ (1–16 μg/kg) to the anaesthetized dog produced dose-related salivation (Fig. 1). The 8 μg/kg dose of $PGF_{2\alpha}$ which was used in other experiments to be described in this report, also produced a weak pressor response of 28 ± 6 mmHg and a negative cardiac chronotropic response of -25 ± 9 beats/min (mean $\pm$ S.E., $n=6$).

Optimal parameters for chorda tympani nerve stimulation were studied in three dogs. Supramaximal voltage was determined in each animal by selecting the voltage required to produce maximal salivation (drops/2 min) from the left submaxillary gland at a stimulation frequency of 6·25 Hz. Once this had been determined, the nerve was stimulated supramaximally for periods of 15 s with square wave pulses of 10 ms duration at frequencies of 6·25, 12·5, 25·0 and 50·0 Hz. The results of this study were averaged and plotted as salivary response versus stimulation frequency. The graph showed a distinct optimal stimulation frequency of 25·0 Hz.

Electrical stimulation of the chorda tympani nerve to the left submaxillary gland produced salivation which occurred within several seconds after initiation of electrical stimulation. In contrast, salivation due to $PGF_{2\alpha}$ occurred only after a latent period of 20–30 seconds. Saliva produced by either procedure appeared to be of a thin, watery consistency. Typical salivary responses to nerve stimulation and $PGF_{2\alpha}$ are shown in Fig. 2. Salivation produced by each procedure was abolished by pretreatment with atropine.

Mean control salivary responses to electrical stimulation of the chorda tympani (supramaximal voltage, 25 Hz, 10 ms duration, for 15 s) and $PGF_{2\alpha}$ (8 μg/kg) for all animals used in this study were 17 ± 1 and 39 ± 3 drops/2 min, respectively.

Initial experiments were conducted to demonstrate the effect of time on the salivary response to nerve stimulation or $PGF_{2\alpha}$. As shown in Table 1, the salivary response to either procedure declined with time, in animals receiving saline infusions.

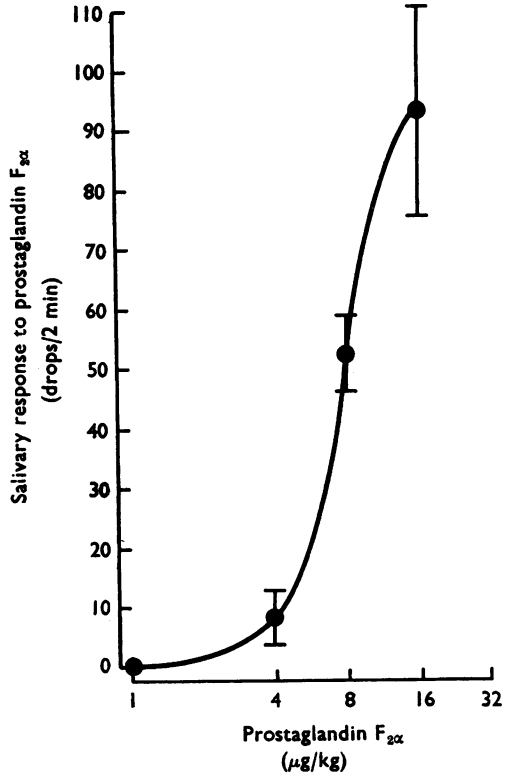

FIG. 1. Relationship between salivary response (drops/2 min) and dose of $PGF_{2\alpha}$ (μg/kg) in the anaesthetized dog. Each value is the mean of 3–6 observations. Vertical bars are standard errors of the mean.

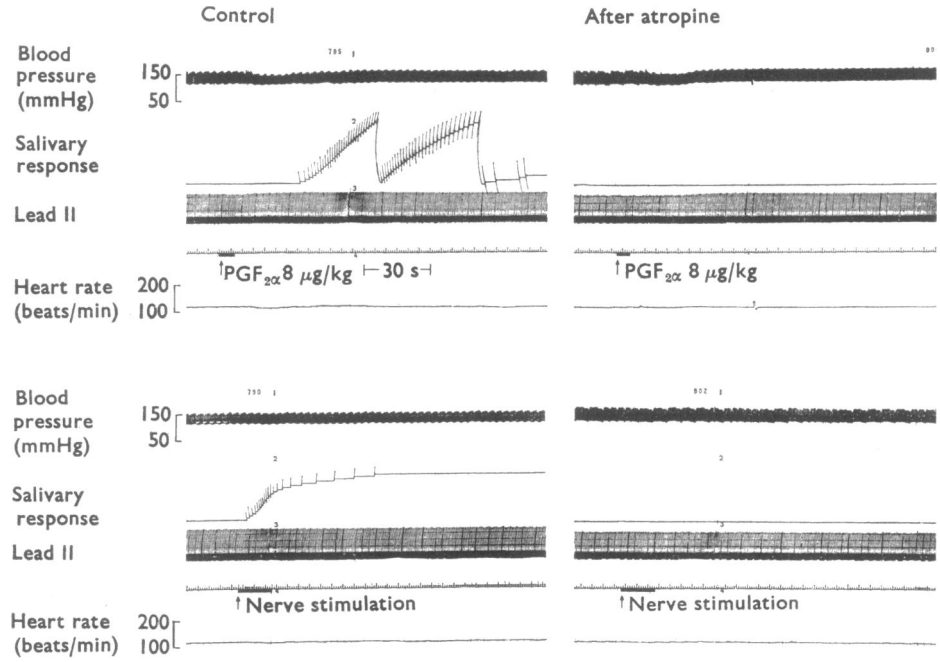

FIG. 2. Experimental tracing illustrating the salivary response to $PGF_{2\alpha}$ (8 μg/kg) and chorda tympani nerve stimulation in the anaesthetized dog. In the salivary response tracing each deflection represents one drop of saliva. Pretreatment with atropine (1 mg/kg) abolished the salivary response to each procedure.

After an elapsed time of 30 min responses to nerve stimulation and $PGF_{2\alpha}$ were 69 ± 11 and $88 \pm 10\%$ of control, respectively. After 60 min the responses were 53 ± 10 and $61 \pm 9\%$ of control.

The effect of blockade of α-adrenoceptors on salivary responses was evaluated in another set of experiments. As shown in Table 1 pretreatment with the α-adrenoceptor blocking agent phentolamine (2 mg/kg) produced effects which were not significantly different from the time-related reductions observed in saline-treated animals. Administration of atropine (1 mg/kg) after phentolamine completely abolished salivation produced by either procedure (Table 1).

The effect of ganglionic blockade on the salivary response to nerve stimulation or $PGF_{2\alpha}$ was evaluated in other animals treated with hexamethonium (10 mg/kg). Table 1 shows that this treatment greatly reduced the salivary response to nerve stimulation ($P<0.05$), but did not significantly alter the response produced by $PGF_{2\alpha}$. Subsequent injection of atropine (1 mg/kg) abolished salivation to $PGF_{2\alpha}$ and the small salivary response to nerve stimulation remaining after hexamethonium treatment.

The results of these experiments suggested that salivation produced by $PGF_{2\alpha}$ did not involve stimulation of ganglia or α-adrenoceptors. The response appeared to be due to an activity on cholinoceptors within the submaxillary gland. To obtain information as to whether $PGF_{2\alpha}$ produced salivation by acting as a direct agonist on cholinoceptors in the gland, or by releasing acetylcholine which would then directly stimulate receptors, animals were pretreated with physostigmine (100 μg/kg). Table 1 shows that after physostigmine the salivary response to nerve stimulation at the 30 min interval was not significantly different from the response observed in saline treated animals. However, the response to nerve stimulation observed after an elapsed time of 60 min in physostigmine treated animals was significantly greater than the response seen in animals receiving saline infusions. Table 1 further shows that after physostigmine treatment salivary responses to $PGF_{2\alpha}$ were greater at both observation intervals, than the responses to $PGF_{2\alpha}$ occurring in saline treated animals ($P<0.05$).

Discussion

The results of this study demonstrate that administration of $PGF_{2\alpha}$ to the anaesthetized dog produces dose-related salivation. Attending the salivary response are weak pressor and negative chronotropic effects of similar magnitude to those previously

TABLE 1. *Effect of various treatments on salivary responses to chorda tympani nerve stimulation and $PGF_{2\alpha}$ (8 μg/kg) in the anaesthetized dog*

Treatment	Salivary response (% of control) 30 min interval		60 min interval	
	Nerve stimulation	$PGF_{2\alpha}$	Nerve stimulation	$PGF_{2\alpha}$
Saline	69·5±11·5*	88·8±10·8	53·1±10·8	61·2±9·66
Phentolamine	69·9±10·8	68·9±13·6	—	—
Phentolamine+atropine	—	—	0·0†	0·0†
Hexamethonium	13·7±8·04†	66·7±9·70	—	—
Hexamethonium+atropine	—	—	0·0†	0·0†
Physostigmine	75·3±8·46	125·3±3·07†	99·2±13·0†	110·2±9·22†

* Mean ± S.E. (four-five observations for each value). † Significantly different from corresponding saline treatment value ($P<0.05$).

reported (Nakano & McCurdy, 1968). Injection of PGE_1 lowered arterial blood pressure and increased heart rate, but did not produce salivation.

Electrical stimulation of the parasympathetic nerve supplying the submaxillary gland produced salivation, the response being observed almost immediately after onset of stimulation. Salivation induced by $PGF_{2\alpha}$, in contrast, was not immediate but occurred only after a latent period of 20–30 seconds. Pretreatment with atropine abolished the salivary response to $PGF_{2\alpha}$ and to nerve stimulation.

To determine the mechanism by which $PGF_{2\alpha}$ produced salivation, several sets of experiments were performed using pharmacological blocking agents. Pretreatment of animals with phentolamine did not reduce salivary responses to nerve stimulation or $PGF_{2\alpha}$ to a greater extent than reductions observed with time in saline treated animals, suggesting that neither response is mediated by α-adrenoceptors. In other animals pretreated with hexamethonium, the reduction in salivary response to $PGF_{2\alpha}$ was not significantly less than the time related decrease in response seen in animals receiving saline infusions, although the response to preganglionic nerve stimulation was significantly reduced (Table 1). These experiments indicate that salivation induced by $PGF_{2\alpha}$ is not the result of ganglionic stimulation. In addition, since the chorda tympani nerve supplying the left submaxillary gland was decentralized (for electrical stimulation), and since neither treatment with phentolamine nor hexamethonium significantly reduced the response to $PGF_{2\alpha}$, salivation due to an effect in the central nervous system seems unlikely. Subsequent injection of atropine after phentolamine or hexamethonium abolished salivation due to nerve stimulation or $PGF_{2\alpha}$. Thus, taken together these results suggested that $PGF_{2\alpha}$ was producing salivation by an action on cholinoceptors in the salivary gland.

A final set of experiments was conducted to determine the effect of physostigmine treatment on the salivary response to $PGF_{2\alpha}$. If $PGF_{2\alpha}$ produced salivation by acting as a direct agonist on cholinoceptors in the gland, then pretreatment with physostigmine would presumably not influence the response since cholinesterase does not appear to be involved in the metabolism of prostaglandins (Ramwell & Shaw, 1971). If, however, $PGF_{2\alpha}$ produced salivation by releasing endogenous acetylcholine then pretreatment with physostigmine should augment the response. As shown in Table 1, pretreatment with physostigmine did result in an increase in salivary response to $PGF_{2\alpha}$, at both observation intervals, and the response to nerve stimulation at the 60 min interval. It should be emphasized that the augmented salivary response to $PGF_{2\alpha}$ after physostigmine treatment is not the result of changes in baseline flow of saliva, since any flow occurring in the 2 min immediately before $PGF_{2\alpha}$ administration would be subtracted from the response obtained to the prostaglandin. Furthermore, at the dose level used in these experiments physostigmine treatment did not result in a baseline salivary flow. Our results therefore suggest that the salivary response to $PGF_{2\alpha}$ in the anaesthetized dog is due, at least in part, to release of acetylcholine from cholinergic nerve terminals.

Recent studies have shown that prostaglandins can markedly influence adrenergic neurotransmission. Hedqvist & Brundin (1969) reported that PGE_1 antagonized the increase in perfusion pressure of the isolated, perfused cat spleen produced by stimulation of the splenic nerve or administration of noradrenaline. Analysis of the splenic effluent revealed that treatment with PGE_1 reversibly decreased noradrenaline overflow following nerve stimulation. These results suggest that PGE_1 is capable of producing effects at both pre- and postsynaptic sites. Kadowitz *et al.*

1971a, 1971b) studied the effect of various prostaglandins on vasoconstrictor responses to sympathetic nerve stimulation and injected noradrenaline, in the dog. They reported that PGE_1 antagonized vasoconstrictor effects of nerve stimulation and noradrenaline, while $PGF_{2\alpha}$ enhanced responses to these procedures. The mechanism by which $PGF_{2\alpha}$ enhanced the response to sympathetic nerve stimulation is not known, but either facilitation of transmitter release or blockade of transmitter reuptake into adrenergic nerve terminals appear likely possibilities. The results of the present study indicate that $PGF_{2\alpha}$ may also interact with cholinergic nerve terminals in a similar manner to alter cholinergic neurotransmission.

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