Differential Effects of Prostaglandin F$_{2\alpha}$ on Oxytocinergic and Non-oxytocinergic Neurones in the Paraventricular Nucleus of the Lactating Rat

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Synopsis

The effects of the prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) given into the third cerebral ventricle on the unit activity of neurosecretory neurones in the paraventricular nucleus (PVN) were studied in urethane-anesthetized rats. The firing activity of PVN neurones was recorded extracellularly and 50 neurones were antidromically identified as neurosecretory neurones. Thirty of them were classified oxytocinergic neurones because they gave a burst of action potential 12-15 sec before reflex milk ejection and the remaining twenty PVN neurones which showed no response prior to reflex milk ejections were regarded as non-oxytocinergic ones.

Twenty-five (83%) of the 30 oxytocinergic neurones increased in the firing rate following the intraventricular (IVT) injection of PGF$_{2\alpha}$ (500ng in 1µl of isotonic saline) and the responses lasted for about 20-30 min. The remaining 5 (17%) oxytocinergic neurones showed no response in the firing rate to IVT PGF$_{2\alpha}$. Fifteen (75%) of the 20 non-oxytocinergic neurones decreased in the firing activity in response to IVT PGF$_{2\alpha}$, and the remaining 5 (25%) of them showed no response. IVT injection of isotonic saline (1µl) did not affect the firing activity of both the oxytocinergic and non-oxytocinergic cells. The intramammary pressure was slightly increased by the IVT administration of PGF$_{2\alpha}$.

These findings indicate that IVT PGF$_{2\alpha}$ has a differential action on oxytocinergic and non-oxytocinergic neurones in rats.

Recent studies have suggested that prostaglandin E$_1$ (PGE$_1$) (Andersson and Leksell, 1975; Leksell, 1976) and PGE$_2$ (Vilhardt and Hedqvist, 1970; Yamamoto et al., 1976) act on the central nervous system to stimulate the release of vasopressin. On the other hand, conflicting results were reported on the effect of PGF$_{2\alpha}$ on oxytocin release; Gillespie et al. (1972) and Cobo et al. (1974) showed that oxytocin release was induced by intravenous administration of PGF$_{2\alpha}$ in women, while Prilusky and Deis (1976) reported that milk ejection was suppressed by intraperitoneal administration of PGF$_{2\alpha}$ in lactating rats, although all of these authors argued that PGF$_{2\alpha}$ acted on the hypothalamo-neurohypophyseal system.

The present experiment is designed to answer the question whether PGF$_{2\alpha}$ acts directly on the central nervous system to affect the neurosecretory neurones, and whether PGF$_{2\alpha}$ exerts a facilitatory or inhibitory action on oxytocinergic neurones, by investigating the effects of PGF$_{2\alpha}$ injected intraventricularly (IVT) on the antidromically identified neurosecretory neurones in the paraventricular nucleus (PVN) of rats.
Materials and Methods

Experiments were performed in 23 lactating rats (270–350 g), on day 7–14 of lactation under urethane anesthesia (1.1 g/kg, i.p.). Unit recording and antidromic identification of neurosecretory cells were made by the method reported previously (Negoro and Holland, 1972). A bipolar electrode was implanted in the posterior pituitary gland for the antidromic identification of neurosecretory cells. Unit activity was recorded extracellularly from PVN through a tungsten microelectrode insulated with Insl-X (Insl-X Product Corp.). A stainless steel double-barrelled cannula was inserted into the third ventricle and fixed to the skull for the administration of PGF$_{2\alpha}$ and isotonic saline. The relative positions of the stimulating electrode, recording electrode and cannula are illustrated in Fig. 1. The stereotaxic coordinates were obtained from the atlas of Albe-Fessard et al. (1966). The teat duct of a mammary gland was cannulated with a polyethylene tube and connected to the pressure transducer for the measurement of the intramammary pressure. The saphenous vein was cannulated with a polyethylene tube for the administration of oxytocin (Syntocinon, Sandoz). In order to induce reflex milk ejections 7–10 pups were applied to the uncannulated nipples. PGF$_{2\alpha}$ (Onoyakuhin Kogyo) was dissolved in isotonic saline at a concentration of 500 μl/ml. Every rat was given 1 μl of isotonic saline (control injection) and 500 ng PGF$_{2\alpha}$ with a microsyringe through the cannula inserted into the third ventricle.

In order to confirm the recording site, lesion was made with anodal DC current of 100 μA for 20 sec. One μl of 2% trypan blue was injected through the cannula for the determination of the site of injection. Animals were sacrificed with an overdose of urethane and were perfused with 10% buffer formalin for the histological verification of the brain.

Fig. 1. Diagrammatic view of the positions of the cannula in the third ventricle, the bipolar stimulating electrode in the neural lobe of the pituitary gland and the recording microelectrode in the paraventricular nucleus. The oscilloscope trace (below right) is a photograph of 5 superimposed sweeps showing a constant latency of the antidromically activated action potentials. Calibration, 1 mV and 10 msec. OCH, optic chiasm; SO, supraoptic nucleus; PV, paraventricular nucleus; AMP, amplifier; CRO, cathode ray oscilloscope; STIM, stimulator.
Results

Identification of oxytocinergic and non-oxytocinergic neurones

Intramammary pressure and firing activity of the neurosecretory cell in the PVN were recorded simultaneously during suckling by 7-10 pups in 23 lactating rats. The reflex milk ejections started 14.0-30.0 min after applying the pups and recurred at an interval of 2.7-19.3 min. Thirty (60%) of 50 PVN neurones which were antidromically identified as neurosecretory cells displayed an acceleration in firing 12-15 sec prior to the milk ejections (Fig. 2A). The acceleration of the PVN units was characterized by a sudden and marked increase in firing from the background activity of 2.0±0.4 spikes/sec (mean±S.E.M.) to peak firing rate of 21.1±3.1 spikes/sec. The duration of the acceleration was 2.6±0.3 sec. Then the increased firing of the unit was followed by after-inhibition, which lasted for 5.0-35.0 sec.

Fig. 2. Effect of PGF$_2$α on neurosecretory neurones identified as oxytocinergic cells. A. Simultaneous recordings of the unit activity of a neurosecretory cell and intramammary pressure during suckling in the lactating rat. Note that the unit in A exhibits a dramatic increase in the firing activity which occurred about 15-18 sec before the onset of milk ejection. B and C. Records of firing rate from two oxytocinergic cells showing the response to 500 ng PGF$_2$α (IVT). B is the record from the same unit as shown in A.
sec. The PVN neurones which showed these characteristics of firing during the reflex milk ejections were classified as oxytocinergic cells. The remaining 20 (40%) PVN neurosecretory cells displayed no response prior to the reflex milk ejections. They were classified as non-oxytocinergic neurones (Fig. 3A).

Effect of PGF$_{2\alpha}$ on oxytocinergic and non-oxytocinergic neurones

The effects of IVT injection of PGF$_{2\alpha}$ (500 ng) on the firing activity of PVN neurones classified as oxytocinergic or non-oxytocinergic cells were examined. The statistical significance of change in the firing rate after the injection was determined in
each neurone as follows; the firing rates (spikes per 10 sec) were measured in an interval of 5 min immediately before the PGF$_{2\alpha}$ injection and in an interval of 3 min between 3 and 6 min after the injection, and the difference in the mean firing rates in the two intervals was tested by t-test.

Twenty-five (83%) of 30 oxytocinergic neurones showed a significant increase (P<0.001–P<0.05) in the firing rate in response to PGF$_{2\alpha}$ (Fig. 2B, 2C). The latency for the initiation of the response was $1.1 \pm 0.2$ min and the firing rate reached a maximum $4.8 \pm 1.2$ min after the injection. The average increase in the firing rate was $60.7 \pm 12.1\%$. The acceleration of the firing lasted for about 20–30 min. The remaining 5 oxytocinergic neurones (17%) showed no significant change in firing after PGF$_{2\alpha}$ injection.

On the other hand, 15 (75%) of the 20 non-oxytocinergic neurones decreased their firing rate significantly (P<0.001–P<0.01) and the remaining 5 neurones (25%) showed no change in response to PGF$_{2\alpha}$ injection. The inhibitory response initiated $1.4 \pm 0.2$ min after PGF$_{2\alpha}$ injection and lasted for about 10 min (Fig. 3B, 3C). The average decrease in the firing rate was $54.4 \pm 6.2\%$.

Isotonic saline injected IVT as the control had no significant effect on the firing activity of oxytocinergic and non-oxytocinergic neurones.

**Effect of PGF$_{2\alpha}$ on intramammary pressure**

Slight increases in the intramammary pressure were observed 40–100 sec after IVT injection of PGF$_{2\alpha}$ and they lasted for nearly 30 min. The peak of the change in the intramammary pressure following PGF$_{2\alpha}$ injection was equivalent to that obtained by the intravenous injection of 40–140 µU oxytocin.

**Discussion**

Wakerley and Lincoln (1973) and Lincoln and Wakerley (1974) have shown that about a half of neurosecretory cells in the PVN and supraoptic nucleus exhibit a distinctive pattern of accelerated firing before the milk ejection response induced by suckling pups. We confirmed their observations in this study. The reflex milk ejection occurs in the absence of detectable release of vasopressin (Wakerly et al., 1973). An immunocytochemical study (Vandesande and Dierickx, 1975) also showed that one half of the PVN neurosecretory cells were oxytocin-containing cells and the other half vasopressin-containing ones. It is, therefore, reasonably assumed that the neurosecretory cells which show the characteristic pattern of firing before the reflex milk ejection are oxytocinergic and that most of the unresponsive neurosecretory cells which are designated as non-oxytocinergic neurones in this paper are vasopressinergic.

It has been generally accepted that under the physiological conditions, hormone-release from the neurohypophysis is triggered by action potentials arriving at the neurosecretory axon terminal. The electrical stimulation of the neurohypophyseal stalk in vivo resulted in the release of hormones (Harris et al., 1969; Wakerley and Lincoln, 1973). Such an effect was abolished if the propagation of impulses was blocked reversibly by radiofrequency current applied below the site of stimulation (Cross and Wakerley, 1974). There is good evidence that neurohypophyseal hormone-discharge is correlated with an increased firing rate of the neurosecretory cell (Dyball, 1971; Dyball and Dyer, 1971; Wakerley and Lincoln, 1973; Lincoln and Wakerley, 1974; Harris et al., 1975; Wakerley et al., 1975; Brimble et al., 1978). In the present study, PGF$_{2\alpha}$ activated 83 per cent of oxytocinergic neurones and inhibited 75 per cent of non-oxytocinergic neurones. Thus it is likely that PGF$_{2\alpha}$ administered IVT stimulates oxytocin release and suppresses vasopressin release. Gillespie et al. (1972) reported
that the intravenous infusion of PGF$_{2\alpha}$ released oxytocin in both women and men. Cobo et al. (1974) also observed PGF$_{2\alpha}$-induced milk ejection in lactating women. Our result obtained in rats is compatible with their findings.

Gillespie et al. (1972) speculated that a release of prostaglandin from the decidua, heralding the onset of labor, would cause a release of oxytocin. On the other hand, prostaglandins (E$_1$, E$_2$, F$_{1\alpha}$ and F$_{2\alpha}$) have been identified in various regions in the brain including the hypothalamus (Holmes and Horton, 1968). The release of prostaglandins from the cerebral cortex was evoked by stimulating peripheral nerves (Ramwell and Shaw, 1966). Therefore, the possibility that PGF$_{2\alpha}$ in the brain acts as a transmitter or modulator for the release of oxytocin should also be considered. However, a natural stimulus that affects release of oxytocin and vasopressin in a reciprocal manner has not been documented. Suckling stimuli (Wakerly et al., 1973) and estrogen (Yamaguchi et al., 1979) are the factors that facilitate oxytocin release exclusively but these stimuli do not suppress vasopressin release. Negoro et al. (1973) showed in the rat that vaginal distension and pinching stimuli either activated or inhibited the activity of PVN units in a certain stage of the reproductive cycle or in a certain hormonal condition. However, since PVN units recorded were not identified to be oxytocinergic or non-oxytocinergic in their study, such differential effects of the stimuli on the PVN unit are hard to be ascribed to the mediation of PGF$_{2\alpha}$ at present. Thus further studies are required to determine whether or not endogenous PGF$_{2\alpha}$ plays a physiological role in oxytocin release.

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References


