CUMULATIVE EFFECT OF PROSTAGLANDIN E2 (PGE2) INJECTIONS ON ELECTROENCEPHALOGRAPHIC ACTIVITY IN MALE MONKEYS (MACACA MULATTA)

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Abstract. Bipolar stainless steel electrodes were stereotaxically implanted in hypothalamic and cerebral cortical regions of 24 adult male gonadally intact monkeys. Electrical activity of these regions was recorded before and after the first, second, third, thirteenth, fourteenth and fifteenth day of intravenous (I. V.) injection of PGE2 (400 μg/kg body weight). The inferences drawn from the EEG record were: (i), the immediate response was a tendency towards spindle formation (ii), high voltage spindles appeared at regular intervals in all sites i.e., preoptic area, posterior hypothalamus, ventromedial nucleus and cerebral cortex (iii), the changes observed in EEG were progressive with time and transitional with respect to various regions of the brain (iv), these changes were synchronized whenever and wherever they were present, but the generalized showing in the form of overall inhibition could never be seen (v), the dose showed a cumulative effect after repeated daily injections of PGE2.

INTRODUCTION

Prostaglandins (1) are one of the important constituents of semen. The neural mechanisms for the regulation of gonadotrophin secretion from the pituitary and sex hormones from the gonads (4, 9) as well as

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sex behavior (19, 22) are located in the hypothalamus. Administration of prostaglandins in rodents has been shown to produce an overall inhibition of spermatogenesis, testicular atrophy, atrophy of the accessory sex organs as well as reduced plasma testosterone level and the presence of large number of exfoliated germ cells in the epididymis (7, 8). Prostaglandins have been shown to act both on the hypothalamus and the pituitary to augment release of gonadotrophins and prolactin (3, 11, 14).

Prostaglandins have a depressant action on behavior, which varies in intensity from mild sedation to catatonia (12). In EEG activity during prostaglandin induced sedation, a slow wave pattern (6, 20) predominates or the EEG is reminiscent of that recorded during the activated sleep (10). Lyneham et al. (16) have reported electroencephalographic abnormalities of epileptic type in some patients treated with PGF$_{2\alpha}$ for termination of pregnancy.

The present investigation was undertaken to study the alterations induced by prostaglandins E$_2$ on EEG activity of mature male monkeys with an aim to accentuate the central neural integration responsible for all the induced changes in the periphery and also to observe a cumulative effect on EEG record after repeated daily injections of PGE$_2$ for 15 days.

**MATERIAL AND METHODS**

Twenty four adult male rhesus monkeys weighing between 4 to 5 kg were procured from T. E. Peterson Ltd., New Delhi. All the animals were housed in individual cages measuring $2' \times 2' \times 2.5'$ and kept in a large animal room, which was moderately temperature controlled and well ventilated. Monkeys were fed with standard Hindustan Levers food pellets and water ad libitum.

For stereotaxic implantation of electrodes, the animals were anesthetized with intraperitoneal injection of sodium pentobarbitol (35 mg/kg b. wt.). Bipolar stainless steel electrodes with their tips scraped about 1 mm were stereotaxically implanted in the preoptic area (PO), posterior hypothalamus (PH), ventromedial nucleus (VMN) and cerebral cortex (CO), using the technique of Anand (1). For cortical EEG studies, watch screws were implanted. When the animals had recovered from the effects of surgical trauma, EEG activity of different areas of the brain after chronic administration of PGE$_2$ was recorded, on polygraph (Inco, India). Electroencephalograph was taken for half an hour prior to and 15, 30, 60, 90, 120 and 150 min after PGE$_2$ (400 µg/kg b. wt.) or vehicle injection, on the 1st, 2nd, 3rd, 13th, 14th and 15th day of treatment. For control observations each monkey served as its own control.
After completion of the experimental procedure, electrode sites were marked by passing direct current of 15 mA for 30 s to produce small iron deposits at the electrode tips. The animals were sacrificed and their brain perfused with 10% formalin solution containing 1% potassium ferrocyanide. This produced a prussian blue reaction with iron deposits resulting in blue spots at the site of electrode tips.

RESULTS

The EEG records were analyzed using a procedure already described (17). The analysis of maximum change in frequency and voltage was made separately and illustrated by the representative tracing of EEG record.

1. Response to the 1st injection: No attenuation of EEG record could be seen after PGE$_2$ injection, rather the activity in PO and VMN was slightly potentiated (Fig. 1). This potentiation was

EFFECT OF PGE$_2$ ON EEG OF HYPOTHALAMUS IN MALE MONKEYS
(FIRST INJECTION)

![Tracings](image)

Fig. 1. Shows the tracings recorded from PO, PH, VMN and CO before and after the 1st injection of PGE$_2$ (400 µg/kg b.wt.) in monkeys. Immediately after injection no specific alterations were observed in EEG activity, rather the activity from PO and VMN was slightly potentiated. After 60 min tendency towards spindle formation could be observed from all areas.

<table>
<thead>
<tr>
<th>Before Injection</th>
<th>After 15 min</th>
<th>After 30 min</th>
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<tbody>
<tr>
<td>A - Prefrontal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B - Posterior Hypothalamus</td>
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<tr>
<td>C - Ventromedial Nucleus</td>
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<tr>
<td>D - Cortex</td>
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AMPLITUDE CALIBRATION: 50 µV
well marked after 30 min while PH also showed increase in voltage. However, after 60 min slight tendency towards spindle formation could be recorded from all areas and the record was comprised of wave bursts of comparatively lower voltage. These bursts appear to have originated in PO and then spread to PH, VMN and CO (Fig. 1). After 90 min the tendency towards spindle formation was quite obvious in VMN.

2. Responses to the 2nd injection: The basal EEG record on the second day prior to the administration of PGE₂ was different from that of the basal record (Fig. 1) of the first day i.e., before the first injection. The basic change was that the whole of the record was slightly amplified in voltage without any change in the frequency of wave pattern (Fig. 2). This pattern of waves persisted for 30 min even after the second injection, while after an interval of another 15 min occasional slow waves started appearing in all areas and were more pronounced in VMN, PH, PO and CO, in that order. At this stage a slight tendency towards spindle formation was observed. However, well developed spindles were seen in recordings from all areas 60 min after the second injection (Fig. 2). The average frequency, voltage and duration of these spindles was 7 cycles per second (cps), 36.5 microvolts (μV) and 1.5 s. These spindles appeared after an interval of 2.9 s while during

![Fig. 2. Tracings showing EEG record after the 2nd injection of PGE₂. Slight increase in voltage was observed without any change in frequency of wave. Tendency towards spindle formation could be seen from all areas which took the full fledged shape after 60 min of PGE₂ injection.](image-url)
this period the average pooled frequencies and voltages of PO, PH, VMN and CO were 4.6, 4.57, 5.01 and 4.25 cps and 31.30, 30.84, 27.34 and 32.76 µv respectively. The spindles were also present at an interval of 90 and 120 min after PGE$_2$ administration. Furthermore, the involvement of additional time factor further potentiated the spindle responses (Fig. 2), which have been beautifully traced out in the recording 150 min after PGE$_2$ administration. There was a tremendous increase in voltage (42.50 µv) of spindle waves and their duration (3 s). Moreover, the frequency of their occurrence was much more augmented. The classical characteristic of these spindles was that whenever and wherever they appeared, the occurrence was synchronous in nature. The frequencies, voltages and duration of occurrence of these spindles were in the range of 4.00–9.00 cps, 28.66–42.50 µv and 0.5 to 2.5 s while the average pooled frequencies and voltages of the record at this time from PO, PH, VMN and CO was 4.46, 4.38, 4.60 and 4.38 cps and 29.82, 39.38, 25.48 and 39.80 µv respectively.

3. Responses to the 3rd injection: All the changes in the EEG wave pattern seen after 2nd injection reoccurred after the 3rd injection as well.

4. Responses to the 14th injection: The EEG record taken prior to the 14th injection demonstrated a certain degree of cumulative effect of continuous daily injections of PGE$_2$. Here along with the tendency towards spindle formation, small duration spindles were also recorded from PO, PH and VMN (Fig. 3). After PGE$_2$ treatment initially (i.e., after 15 min) instead of spindles, high voltage bursts of slow waves were recorded (Fig. 3) which took the shape of paroxysmal bursts. These paroxysmal bursts appear to have been initiated from PO and later had spread to PH, VMN and CO. They were of maximum potentiation from PH and minimum from PO. The paroxysmal waves were comprised of 3–6 cps and 30–43 µv and lasted for 4–16 s. They reappeared after an interval of 11 s. The average pooled frequencies and voltages at this time from PO, PH, VMN and CO were 4.77, 4.60, 4.80 and 4.57 cps and 21.84, 33.00, 36.16 and 20.84 µv respectively.

5. Responses to the 15th injection: All the changes observed after the 14th injection were repeated even after the 15th injection. The classical representation was that of cumulative effect of PGE$_2$, which was recorded on the 15th day prior to the injection (Fig. 3). The characteristic nature of the spindles when evaluated with respect to their frequency, voltage and duration was exactly similar to the one recorded on the 2nd day at an interval of 150 min (Fig. 2 and 3). The amplitude of spindles was much higher in Fig. 3, i.e., 14 days after PGE$_2$ injection.
The present observations made on EEG agree with the proposal made earlier (7) that prostaglandins have a role to play in hypothalamic mechanisms. Moreover, the hypothalamic areas which are involved in the regulation of gonadotrophin secretion (5, 15) have shown EEG changes in response to PGEs.

The changes observed in EEG activity suggested that intravenously administered PGs crossed the blood brain barrier and affected the brain. The responses to i.v. injections of prostaglandin for 15 days did not
show attenuation of EEG record. This could be attributed to the mode of administration of PGs which might have resulted in quick degradation and excretion of the compound injected.

The repeated occurrence of spindles from PO, PH, VMN and CO and the accentuation of their frequency duration and amplitude suggested that while maintaining the basal activity of EEG, PGs could inhibit the neuronal mechanism for a short span of time. Later, the resemblance of these spindles to seizures suggested simultaneous excitatory action.

The induction of sedation (and/or sleep) on chronic administration of PGE₂, A₁ and F₂ₐ into various anterior and posterior hypothalamic nuclei (21) was not reproducible in the present dose used. This might be due to the difference in the species, route and dose of PGE₂ administered. Due to the existence of a very efficient blood brain barrier for PGs, the effects observed on EEG are obviously the consequences of very small quantities of PGs, which ultimately happen to reach the hypothalamic and cortical areas and have selective excitatory or inhibitory actions. Such an observation made on the brainstem neurons with these compounds (2) suggested that PGs might be the neurotransmitters or they might have some other functions related to transmission in the brain (23).

Injections of PGE₂ produced a cumulative effect, in spite of the fact that the half life of the injected PGE₂ has been shown to be very short (13). It is possible that the actions of prostaglandins of PGE series might be limited by their rapid inactivation. But certain breakdown products of PG are more resistant to metabolism and can be more biologically active (13). Alternatively, PGs like steroidal hormones (17, 18) might be activating various biochemical changes or events which later on could be self-perpetuating.

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