REVIEW

Prostaglandins and Central Serotonergic Activity in the Rat

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Abstract: Pharmacological and biochemical studies indicate that prostaglandins (PGs) exert a modulatory influence on rat brain serotonergic activity. With several experimental approaches, it has been shown that PGEs and PGD_2 facilitate central serotonergic activity in rats. On the contrary, $PGF_{2\alpha}$ not only inhibits rat brain serotonergic activity but also antagonizes the facilitatory effect of the other PGs. The studies support the proposed neuromodulatory role for PGs in central synaptic transmission.

The finding two decades ago that prostaglandins (PGs) are present in the mammalian brain led to the retrospective identification of several biologically active substances isolated from brain tissue and cerebrospinal fluid. Later studies indicated that a wide variety of arachidonic acid metabolites were present in the central nervous system (CNS). Of these, PGs of the E and F series were thought to be the major eicosanoids. More recently, it has been shown that considerable species variation exists in the distribution of central PGs and that PGD_2 is by far the most dominant PG in the rat and mouse brain, the levels of PGE_2 and $PGF_{2\alpha}$ being significantly lower (1–6). In the CNS, as in other organs, PGs are generated *de novo* upon stimulation. There is compelling evidence that PGs are synthesized at multiple sites in the CNS, both neural and non-neural, and complete systems for the biosynthesis and metabolism exist. In addition to enzymatic degradation, the concentration of brain PGs are controlled by choroidal and extrachoroidal carrier-mediated transport processes (1–6).

A variety of physiological functions have been attributed to brain PGs. They have also been implicated in several neurological and psychiatric disorders (1–6). However, the precise role of PGs in pathophysiological states of the CNS remains equivocal. There is considerable experimental evidence that suggests that PGs function as modulators of central synaptic transmission similar to their proposed role as modulators of peripheral sympathetic neurotransmission (7). Direct electrical stimulation as well as stimulation of afferent neural pathways release PGs from many types of CNS preparations, and there is generally a good correlation between the level of neuronal activity and the generation of PGs. Norepinephrine (NE), dopamine (DA) and serotonin stimulate PG synthesis in the brain as well as their release. Conversely, the release of NE and DA from synaptosomal preparations and from cortex and neostriatum is inhibited by PGEs and facilitated by PG synthesis inhibitors. It has also been proposed that PGEs modulate noradrenergic neurotransmission by inhibiting the synthesis of adenosine 3',5'-monophosphate (cyclic AMP), which has been implicated in the mediation of postsynaptic effects of several neurotransmitters, including NE and DA (5, 6). Further, PGE_1 has been reported to enhance rat brain cholinergic (8) and serotonergic (9) activity.

Unlike the role of PGs in central catecholaminergic activity, little attention has been paid to a possible modulatory effect of PGs on central serotonergic activity. The present review concerns work done on this aspect during the last decade in this laboratory. Unless otherwise mentioned, the investigations were conducted on Wistar strain albino rats.

Pharmacological Studies

1. PG Effects on Serotonin-Mediated Drug Actions

The studies were conducted with PGE_1 and $PGF_{2\alpha}$. Morphine analgesia, which was earlier shown to be serotonin-mediated (10) was found to be potentiated by PGE_1 and inhibited by $PGF_{2\alpha}$ and PG synthesis inhibitors (11, 12). Of the PG synthesis inhibitors used, diclofenac was the most potent and longest acting, whereas paracetamol was the least potent and shortest acting. Indomethacin, mefenamic acid and ibuprofen occupied an intermediate position both in potency and in the duration of action (12). In a clinical study, pretreatment of patients with either indomethacin or ibuprofen for three preoperation days, significantly increased the analgesic dose requirement of morphine during the postoperative period. Furthermore, the duration of morphine analgesia in these patients was significantly less as compared to patients receiving only morphine (13).

Morphine-induced catalepsy (14), hexobarbitone hypnosis (15) and anticonvulsant action of phenobarbitone (16), were all shown to be serotonin-mediated effects and were all potentiated by PGE_1 and inhibited by $PGF_{2\alpha}$ and PG synthesis inhibitors (14, 17, 18).

Some pharmacological actions of cannabis resin (delta-9-tetrahydrocannabinol content 12–15%), namely analgesia (19), catalepsy (20) and anticonvulsant action (21), were found to be serotonin-mediated responses and were potentiated by PGE_1 and inhibited by $PGF_{2\alpha}$ and PG synthesis inhibitors (22). On the other hand, pentylenetetrazol-induced convulsions, were inhibited by pharmacologic treatments enhancing central serotonin (23) and by PGE_1, while $PGF_{2\alpha}$ reversed PGE_1-induced inhibition of the convulsions (24).

2. PGE_1-Induced Potentiation of Drug Actions

As indicated earlier, PGE_1 potentiated several serotonin-mediated drug actions. PGE_1-induced potentiation was first studied with sub-effective doses of morphine, hexobarbitone, phenobarbitone and cannabis. Thereafter, 5,6-dihydroxytryptamine (DHT), a selective central neuronolytic agent for serotonergic neurones, and *p*-chlorophenylalanine (PCPA), a

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specific inhibitor of serotonin biosynthesis, were used to support the hypothesis that PGE_1-induced potentiation of morphine analgesia (11), hexobarbitone hypnosis (17), anticonvulsant actions of phenobarbitone (18) and cannabis (22), and analgesic and cataleptic actions of cannabis (22) were serotonin-mediated effects. $PGF_{2\alpha}$ antagonized PGE_1-induced potentiation of these drug actions (22, 31).

3. Antinociceptive and Cataleptic Actions of PGE_1

PGs of the E series are generally regarded as nociceptive agents, stimulating pain receptors in the periphery directly or by sensitizing them to the action of other algogenic agents. The analgesic action of aspirin-like agents has been attributed, at least partly, to their ability to inhibit PG synthesis (25). Unlike aspirin-like analgesics, morphine has been reported to stimulate PG synthesis (26). Even so, the observation that PGE_1 potentiates morphine analgesia (11) was quite unexpected and contradicted an earlier report (27) in which PGE_1 was shown to inhibit morphine analgesia in Sprague-Dawley rats. Later investigations showed that PGE_1 produced significant antinociceptive effect in rats after *i. p.* administration (28). The antinociceptive effects of equi-analgesic doses of morphine (7.5 mg/kg, *i. p.*) and PGE_1 (2.0 mg/kg, *i. p.*) were inhibited to similar extents by DHT and PCPA, indicating that they were serotonin-mediated responses. PGE_1 also induced significant antinociception when administered intracerebroventricularly *(i.c.v.)* against nociceptive impulses induced by radiant heat, pressure and high frequency electric current (29), in doses ranging between 2.5 to 20 μg/rat. Higher doses tended to induce catalepsy. Centrally administered PGE_1 also potentiated the antinociceptive action of morphine (29). PGE_1-induced catalepsy was found to be, at least in part, a serotonin-mediated effect (30). $PGF_{2\alpha}$ inhibited both PGE_1-induced antinociception (28, 31) and catalepsy (30).

4. PGs and Restraint Stress

Stress by restraining was shown to significantly enhance rat brain PGE_2 and $PGF_{2\alpha}$ levels (32) as well as to increase hypothalamic and whole brain concentrations of serotonin (33). Restraint stress-induced autoanalgesia was inhibited by PG synthesis inhibitors as well as by central serotonolytic agents (34). PG synthesis inhibitors significantly attenuated restraint stress-induced potentiation of hexobarbitone hypnosis (35), anticonvulsant effects of phenytoin, phenobarbitone and cannabis resin (36, 37), analgesic effects of morphine (38) and cannabis resin (39), and cataleptic actions of morphine (40) and cannabis (41). Each of these restraint stress-drug interactions was shown to be serotonin-mediated. Since PGs have been envisaged as the first mediator of stress (42), it is likely that the pharmacologic effects of restraint stress are the consequence of PG modulation of central serotonergic activity, as discussed later. It is interesting to note that PG synthesis inhibitors antagonize the increase in rat brain serotonin that is induced by restraint stress (43).

5. PGD_2-Induced Potentiation of Drug Actions

Since PGD_2 is now known to be the major PG in the rat brain (44) and it appears to share some of the central actions of PGEs (45), investigations were conducted on PGD_2-serotonin mediated drug interactions. PGD_2 (0.5, 1.0 and 2.0 mg/kg *i. p.*) produced a dose-related potentiation of hexobarbitone hypnosis (46) and of the anticonvulsant actions of phenobarbitone and phenytoin (47). In either case the potentiation was shown to be serotonin mediated. Furthermore, PGD_2 has been found to potentiate morphine analgesia and to exert *per se* antinociceptive and cataleptic actions on central administration, the effects being mitigated by pharmacological agents reducing central serotonergic activity (unpublished data).

It may be argued that the doses of the PGs used, particularly on *i. p.* administration, ranging from 0.2 to 2.0 mg/kg, are relatively large and could lead to unphysiological concentrations of PGs in the brain. However, it is known that 80 % of injected PGs are removed by the liver and 95 % by the lungs, so that only minute fractions actually reach the brain (9).

Biochemical Studies

Biochemical investigations were conducted to provide corroborative evidence for the pharmacological data. Rat brain serotonin was estimated by a fluorometric technique (48), and the rate of accumulation of serotonin was studied in pargyline or tranylcypromine treated rats (49). The rate of accumulation of serotonin measured by this technique gives an estimate of the rate of synthesis and hence the turnover of the amine (49).

PGE_1 (2.0 mg/kg, *i. p.*) significantly increased serotonin concentrations of whole brain, forebrain, midbrain, pons-medulla and spinal cord (50). On the contrary the PG synthesis inhibitors, diclofenac and paracetamol, significantly decreased steady state levels of the amine (50). PGE_1 (0.4 mg/kg, *i. p.*) significantly enhanced the rate of accumulation of serotonin (51). On the other hand $PGF_{2\alpha}$ (0.5–2.0 mg/kg, *i. p.*) produced a dose related decrease in rat brain serotonin concentrations and reduced the rate of accumulation of the amine (52). PGD_2, like PGE_1, produced a dose-related (0.2–1.0 mg/kg) increase in rat brain serotonin levels. PGD_2 (1.0 mg/kg, *i. p.*) significantly enhanced serotonin concentrations of cortex, hypothalamus, midbrain, pons-medulla and spinal cord, and increased the rate of accumulation of the amine in tranylcypromine-treated rats (53). Further, PG synthesis inhibitors were found to inhibit the increase in rat brain serotonin concentrations induced by morphine (50), cannabis resin and restraint stress (43).

Apart from their postulated role as modulators of the reproductive endocrine system (5), PGs have been proposed as modulators of central synaptic transmission similar to their effects on the peripheral nervous system. PGEs are known to inhibit sympathetic neurotransmission by decreasing the release of NE. On the contrary, $PGF_{2\alpha}$ and PG synthesis inhibitors enhance NE release and facilitate sympathetic transmission (7). PGs of the D type, like PGEs, are known to depress sympathetic neurotransmission (54). Direct and indirect evidence suggests that PGs may also modulate central noradrenergic and dopaminergic activity. However, the data are equivocal (5, 6).

Our investigations indicate that PGs function as modulators of serotonin activity in the rat brain. This conclusion is based on the following observations:

a. PGE_1 and PGD_2 increase rat brain serotonin levels and turnover, while $PGF_{2\alpha}$ has the opposite effect. PG synthesis inhibitors decrease serotonin concentrations and inhibit drug and stress induced increases in brain serotonin levels.

b. PG synthesis inhibitors and $PGF_{2\alpha}$ attenuate several serotonin-mediated drug actions that are potentiated by PGE_1 or PGD_2.

c. PGE_1 or PGD_2-induced potentiation of the action of several centrally acting drugs were antagonized by pharmacological

agents depleting central serotonin. $PGF_{2\alpha}$ also antagonized PGE_1-induced potentiation of these drugs and the inhibition of pentylenetetrazol convulsions.

d. The antinociceptive and cataleptic effects of PGE_1 and PGD_2 were serotonin-mediated actions. $PGF_{2\alpha}$ inhibited these pharmacological actions of PGE_1.

e. PG synthesis inhibitors attenuated restraint stress-induced autoanalgesia and potentiation of the actions of several centrally acting drugs. Restraint stress enhanced rat brain PG concentrations.

Thus, the findings uniformly indicate that the PGs of the E and D series facilitate rat brain serotonergic activity, possibly by enhancing the synthesis and release of the amine, as shown by the turnover studies. On the contrary, $PGF_{2\alpha}$ attenuates rat brain serotonergic activity, possibly by decreasing the synthesis or release of the amine. The optimal functioning of the serotonergic system would thus depend upon the relative activities of these opposing groups of PGs. Interconversion between PGE_2 and $PGF_{2\alpha}$ is known to occur (55) and may be a possibility in the CNS as well. This makes the modulatory role of PGs in central serotonergic transmission a more attractive proposition, since the brain can determine the relative need for a particular PG at a given time. However, further studies in rats and other species are required before a modulatory role for PGs in central serotonergic activity can be conclusively proven.

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RESEARCH ARTICLES

Effect of Dextran on the Release of Gonadotropin-Releasing Hormone (GnRH) Injected into Rats: Plasma GnRH and Gonadotropin Response

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Abstract: Prolonged release of the peptide gonadotropin-releasing hormone (GnRH) from its aqueous solution was achieved by addition of the polymer dextran ($\overline{M}_w \sim 500{,}000$). This effect observed in an *in vitro* system was caused by a decrease of the diffusion coefficient of the peptide. When GnRH was intramuscularly injected into male rats, the addition of dextran to the injected peptide solution led to a prolongation of the GnRH plasma level at the expense of its peak value. This change can be explained by a decrease of the absorption rate of GnRH into blood, which parallels the *in vitro* observation. As a result, the gonadotropin response to GnRH was stronly increased.

Since the identification, characterization, and synthesis of gonadotropin-releasing hormone (GnRH) (1), the hypothalamic decapeptide controlling pituitary gonadotropin secretion, an increasing number of potential uses of this hormone and its superactive agonist analogs has been revealed [reviewed in (2, 3)]. The discovery that GnRH and its agonist analogs not only stimulate but also can inhibit the pituitary-gonadal system by chronic treatment at higher doses [reviewed in (4)] has led to an increasing interest in this peptide. GnRH has recently been proposed as a contraceptive and antitumor agent because of its inhibiting effect on gonadal steroidogenesis (2, 3).

As with other peptides, the therapeutic potential of GnRH is limited by its low metabolic stability, resulting in a very short biological half-life (5, 6), which also applies to the more enzyme-resistant but still degradable analogs (6–8). When GnRH is administered intramuscularly to rats, its plasma level reaches a peak value within a few minutes and then rapidly declines. To prolong an effective plasma level of the peptide, the absorption rate of the peptide from the site of administration into the blood vessel should be lowered.

The aim of this study was to test if a polymer in a dosage form of a peptide, such as GnRH, can retard the release of the peptide. For this purpose we studied (i) the influence of the polymer Dextran T 500 on the diffusion of GnRH out of its solution *in vitro* and (ii) compared this with the *in vivo* effect of the polymer by intramuscularly injecting GnRH solutions with and without the polymer into male rats and measuring the plasma GnRH. Because the polymer led to a prolongation of the GnRH plasma level in these experiments, the plasma levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), representing the primary biological response to GnRH, were also measured.

Materials and Methods

Chemicals

GnRH was a product of VEB Berlin-Chemie, GDR. [His-^{3}H]GnRH with 0.26–0.56 TBq/mmol (7–15 Ci/mmol) was obtained from our institute (9). Dextran T 500 ($\overline{M}_w \sim 500{,}000$) was from Pharmacia, Sweden.

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