

CENTRAL CARDIOVASCULAR AND THERMAL EFFECTS
OF PROSTAGLANDIN D₂ IN RATS¹

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ABSTRACT

Prostaglandin D₂ (PGD₂) is the most common prostaglandin type of the rat brain. Recently a neuromodulator role for PGD₂ has been suggested. In the present work the central cardiovascular and thermal effects of PGD₂ were studied in urethane-anaesthetised rats. When administered at the doses of 0.001-10 µg/rat into the lateral cerebral ventricle (i.c.v.), PGD₂ slightly increased the blood pressure, heart rate and body temperature. The highest dose caused also an initial hypotensive effect. Upon intravenous injections PGD₂ (0.1-10 µg/rat) initially decreased and then weakly increased the blood pressure but had only negligible effects on heart rate and body temperature. Central pretreatment with sodium meclofenamate or indomethacin (1 mg/rat i.c.v.) antagonised effectively all the recorded central effects of PGD₂.

The central cardiovascular and thermal effects of PGD₂ were much weaker than those obtained earlier with other prostaglandins, such as PGF₂α and PGE₂. Therefore, in spite of its abundance in the brain PGD₂ may not be very important for the central cardiovascular and thermal regulation in the rat.

INTRODUCTION

Prostaglandin D₂ (PGD₂) is the most common prostaglandin type in the brain of the rat (2), but its possible physiological functions in the central nervous system are not known. Some investigators have suggested that prostaglandins may be involved in the central control of the cardiovascular and thermoregulatory systems (3,4,5). The highest activity of the PGD synthetase in the rat brain has been found in the hypothalamus and thalamus (6), the brain areas important for cardiovascular (7) and thermal (3,8) homeostasis. Prostaglandins of the E-type are known to be highly active hyperthermic agents in the brain (8,9). In addition, central administration of PGE₂ or PGF₂α induce strong dose-related increases in blood pressure, heart rate and body temperature of the urethane-anaesthetised rat (1,4). Recently it has been reported that PGD₂ at a dose of 10 µg/rat raise the blood pressure and heart rate, when administered intracerebroventricularly (i.c.v.) to the urethane-anaesthetised rat (10). However, only two doses were used and therefore the dose-response relationships are not known.

¹ A preliminary report of this work has been presented at the XXXII Meeting of the Scandinavian Pharmacological Society (1).

In the present study increasing doses of PGD₂ were administered i.c.v. or intravenously (i.v.) to the urethane-anaesthetised rat in order to obtain complete simultaneous cumulative dose-response curves for blood pressure, heart rate and body temperature. Since sodium meclofenamate interferes with the effects of exogenous prostaglandins both in the peripheral tissues (11,12) and in the brain (4,13), it seemed worthwhile to investigate the influence of this agent on the central effects of PGD₂. For comparison, the effect of indomethacin, an equally effective inhibitor of prostaglandin synthesis as sodium meclofenamate (14), was also studied.

MATERIALS AND METHODS

Male Wistar rats (260-360 g) were used. The rats were accommodated to standard ambient conditions for at least one week before the experiments. The lights were on from 6 a.m. to 6 p.m. and the room was completely dark during the remaining 12 hours. The temperature was kept at 22 C and the relative humidity at 40%. The rats received standard rat pellets (Hankkija Oy., Helsinki) and tap water *ad libitum*.

The rats were anaesthetised with urethane (1.5 g/kg intraperitoneally). The trachea was cannulated with a polyethylene tube and the rats were allowed to breathe spontaneously. The mean arterial blood pressure was measured from the left femoral artery by means of a pressure transducer (Hewlett Packard 1280). The heart rate was calculated from the pulse waves by means of a rate computer (Hewlett Packard 8812 A). The left femoral vein was cannulated for intravenous injections. The rats were mounted in a stereotaxic instrument and tilted caudally so that the body formed an angle of 10 degrees with the horizontal plane. Intracerebroventricular injections were performed as described by Paakkari(15). Briefly, an injection needle was introduced into the right lateral ventricle of the brain. A polyethylene catheter, filled with the drug or control solution to be infused, was attached to the needle and the desired amount of the solution was allowed to flow slowly by virtue of the hydrostatic pressure. The proper position of the needle tip was ascertained at the end of each experiment by an injection of dye (Giemsa Solution, Merck) into the cerebral ventricle. The body temperature was measured rectally with a temperature recorder (ELLAB instruments, type TE 3, Copenhagen), a probe being introduced 5 cm into the rectum. A 60 W heating lamp was placed 20 cm above the rat. This distance of the heating lamp was adequate to keep the body temperature of the control rats at 36.9 ± 0.3 C (mean \pm s.e.m.) in an ambient temperature of 22 C.

Administration of drugs

Prostaglandin D₂ (PGD₂), kindly supplied by Dr. J. Pike of the Upjohn Companies, was dissolved in absolute ethanol and stored at -20 C. Further dilutions were made freshly each day in a modified Krebs-Ringer bicarbonate buffer (4) for i.c.v. injections and in 0.9 % (w/v) NaCl (saline) for i.v. injections. The i.c.v. injections were performed in a volume of 10 μ l each and the i.v. injections in a volume of 0.15 ml each. The control animals received the same volume of the appropriate vehicle in each case. Increasing doses of PGD₂ were administered i.c.v. at 20

min intervals in order to obtain cumulative dose-response curves. This interval was chosen because in the preliminary experiments (6 rats for each dose) the maximum tachycardic and hyperthermic effects of PGD₂ were reached 10-20 min after each i.c.v. injection. The maximum pressor effect, however, became apparent 5 min after each i.c.v. injection. For comparison, the i.v. injections were also repeated at 20 min intervals to make sure that the effects induced by centrally administered PGD₂ were not due to a leakage of the drug into the peripheral circulation.

Indomethacin (Orion Pharmaceutical Co., Helsinki) was dissolved in 0.25 N NaOH and sodium meclofenamate monohydrate (Parke, Davis & Co.) in saline. The effect of central pretreatment with indomethacin or sodium meclofenamate on the central actions of PGD₂ was investigated by injecting these drugs i.c.v. 20 min before commencement of the administration of increasing doses of PGD₂ i.c.v. The control animals received the same volume of saline i.c.v. (both of the drug solutions were at the pH of 6.8-7.3).

The Student's t-test was used to calculate the statistical significance of the differences between the control and experimental groups.

RESULTS

Effects of prostaglandin D₂ in non-pretreated rats

Intracerebroventricularly, PGD₂ at the doses of 0.001-1 µg/rat induced rises of blood pressure. The maximum pressor effect was reached 5 min after each i.c.v. injection. The highest dose of 10 µg/rat caused an initial hypotensive effect with its maximum at 1-2 min after each injection. Intravenous administration of PGD₂ (0.1-10 µg/rat induced initial falls in blood pressure followed by slight increases in blood pressure. The maximum hypotensive effect was reached 1-2 min after each i.v. injection. (Fig. 1)

PGD₂ induced a dose-related tachycardic effect, when administered at the doses of 0.001-10 µg/rat i.c.v. The maximum effect was reached 10-20 min after each i.c.v. injection. Intravenously, the same doses slightly increased the heart rate. (Fig. 2)

PGD₂ i.c.v. (0.001-10 µg/rat) increased dose-dependently also the body temperature. The maximum hyperthermic effect was reached 20 min after each i.c.v. injection. Intravenously, the same doses caused only negligible increases in body temperature. (Fig. 3).

Effects of indomethacin and sodium meclofenamate

Centrally administered indomethacin or sodium meclofenamate (1 mg/rat i.c.v.) had no significant effects of their own on the blood pressure, heart rate or body temperature of urethane-anaesthetised rats. There were thus no statistically significant differences in the baseline levels of blood pressure, heart rate and body temperature between indomethacin, sodium meclofenamate and vehicle pretreated groups before commencement of the administrations of PGD₂ i.c.v. (see Fig. 4).

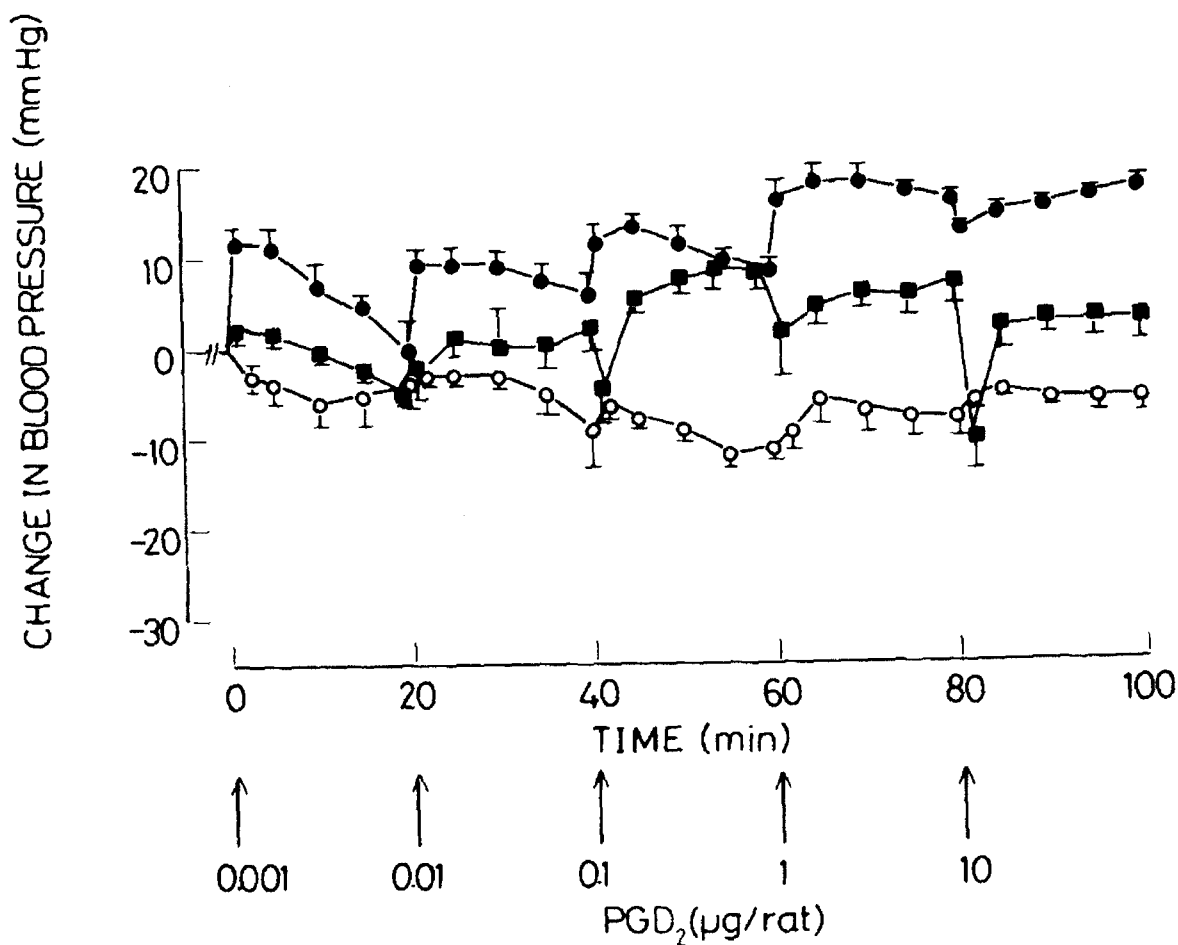


Figure 1. Time-dose-response effect of PGD₂ on blood pressure in urethane-anaesthetised rats. Increasing doses of PGD₂ were administered i.c.v. (●—●) or i.v. (■—■) at 20 min intervals. Open circles represent the vehicle i.c.v. controls. The initial blood pressure level (mean \pm s.e.m.) before commencement of the vehicle or PGD₂ administrations was 110 ± 10 mm Hg in the control group, 103 ± 7 mm Hg in the PGD₂ i.c.v. group and 109 ± 4 mm Hg in the PGD₂ i.v. group. The differences between the initial levels of the groups are not statistically significant. The PGD₂-induced hypertensive effect at the i.c.v. doses of 0.001-10 μ g/rat is significant at the $p < 0.05-0.001$ level as compared to the control values. The changes in blood pressure induced by PGD₂ i.v. (0.1-10 μ g/rat) are significant at the $p < 0.05-0.005$ level as compared to the control group. The differences in the changes in blood pressure between the i.c.v. and i.v. groups at the doses of 0.1-10 μ g/rat are significant at the $p < 0.05-0.005$ level. Vertical bars indicate s.e.m. Each group comprised 6 rats.

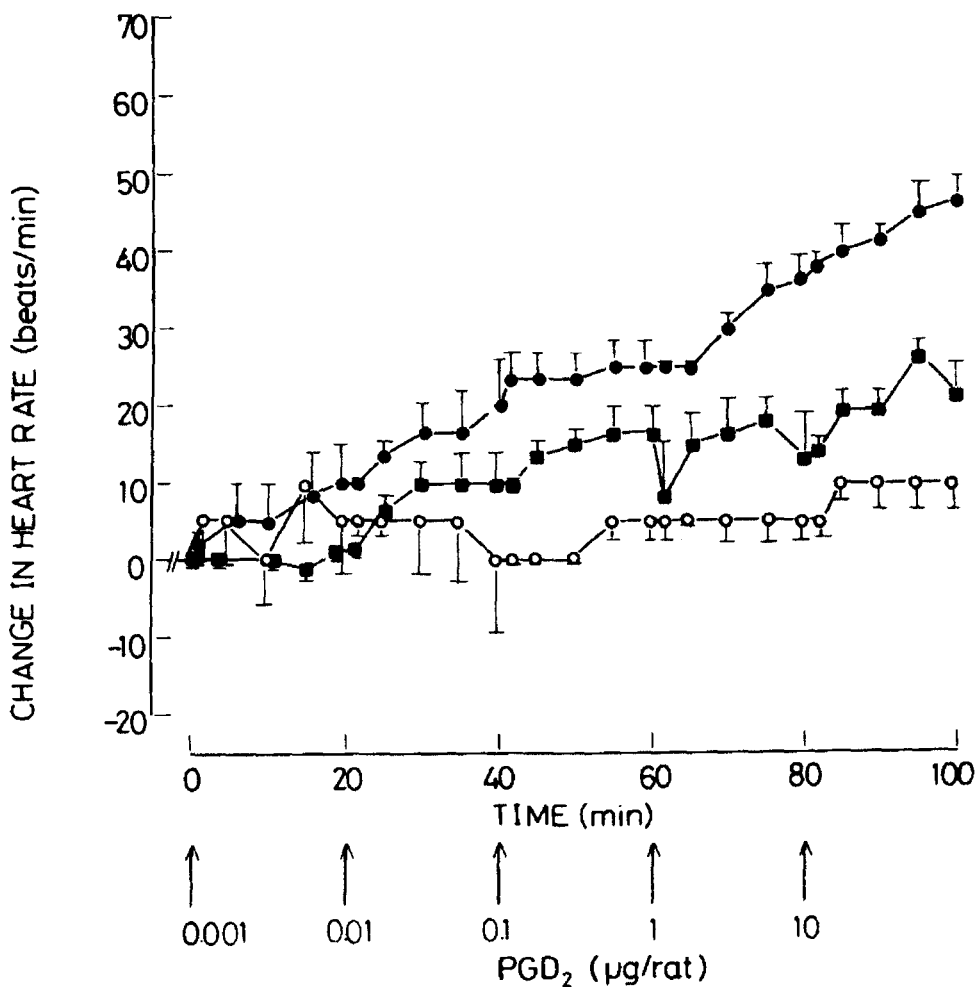


Figure 2. Time-dose-response effect of PGD₂ on heart rate in urethane-anaesthetised rats. Increasing doses of PGD₂ were administered i.c.v. (●—●) or i.v. (■—■) at 20 min intervals. Open circles represent the vehicle i.c.v. controls. The initial heart rate level (mean \pm s.e.m.) before commencement of the vehicle or PGD₂ administrations was 445 ± 10 beats/min in the control group, 445 ± 15 beats/min in the PGD₂ i.c.v. group and 445 ± 5 beats/min in the PGD i.v. group. The tachycardic effect of PGD₂ i.c.v. (0.001-10 μ g/rat) is significant at the $p < 0.05-0.001$ level as compared to the control values. The changes in heart rate induced by PGD₂ i.v. at the doses of 0.001-10 μ g/rat are significant at the $p < 0.05-0.005$ level as compared to the control values. The differences in the PGD₂-induced changes between the i.c.v. and i.v. groups are significant at the $p < 0.05-0.005$ level (0.001-10 μ g/rat). Vertical bars indicate s.e.m. Each group comprised 6 rats.

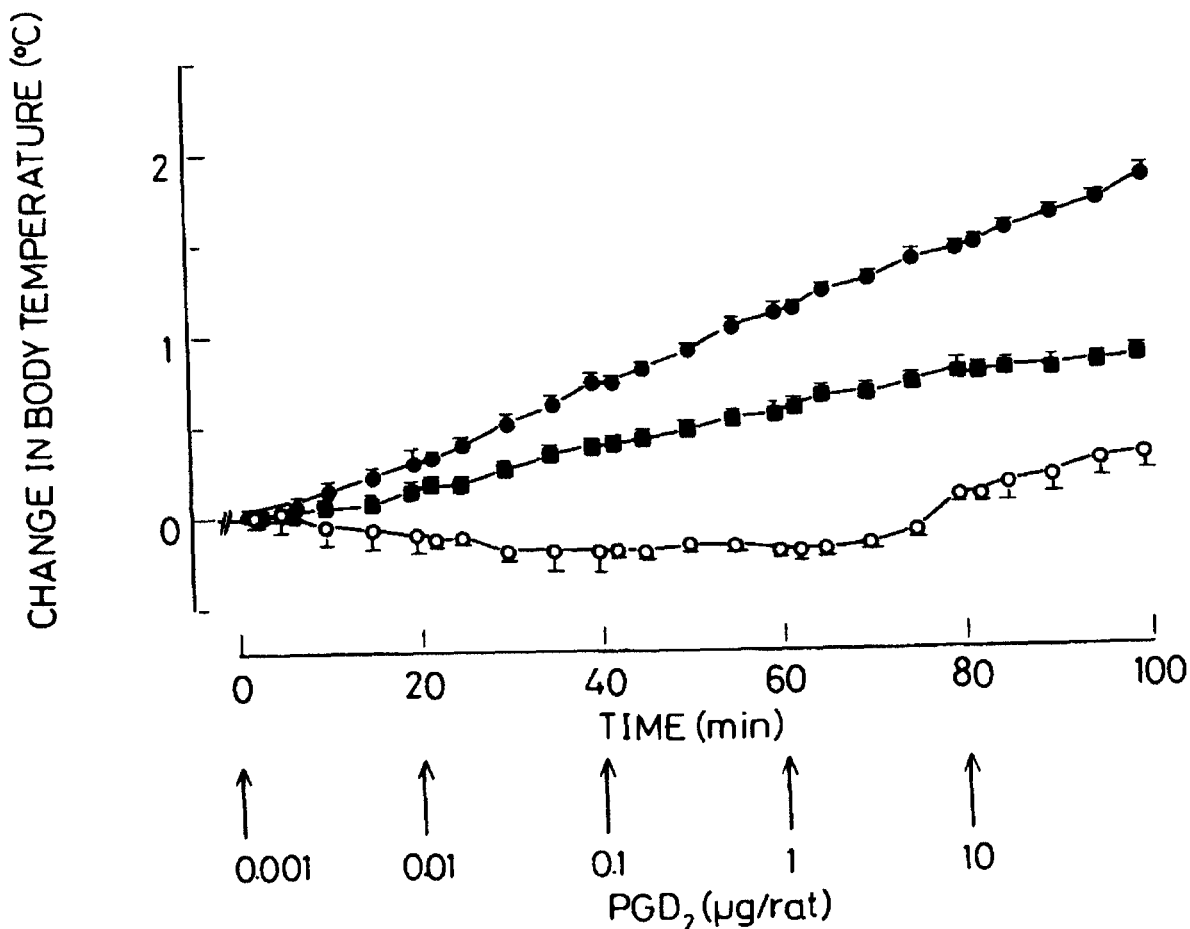


Figure 3. Time-dose-response effect of PGD₂ on body temperature in urethane-anaesthetised rats. Increasing doses of PGD₂ were administered i.c.v. (●—●) or i.v. (■—■) at 20 min intervals. Open circles represent the vehicle i.c.v. controls. The initial body temperature level (mean ± s.e.m.) before commencement of the administrations of vehicle or PGD₂ was 36.3 ± 0.2 °C in the control group, 35.7 ± 0.2 °C in the PGD₂ i.c.v. group and 35.8 ± 0.2 °C in the PGD₂ i.v. group. The differences between the initial levels of the groups is significant at the p<0.05 level. The rises of body temperature induced by PGD₂ i.c.v. (0.001-0.01 µg/rat) are significant at the p<0.05-0.005 level and at the doses of 0.1-10 µg/rat at the p<0.001 level as compared to the control values. The changes induced by PGD₂ i.v. are significant at the doses of 0.001-10 µg/rat at the p<0.05-0.001 level as compared to the control group. Differences in the changes in body temperature between the i.c.v. and i.v. groups are significant at the doses of 0.1-10 µg/rat at the p<0.05-0.005 level. Vertical bars indicate s.e.m. Each group comprised 6 rats.

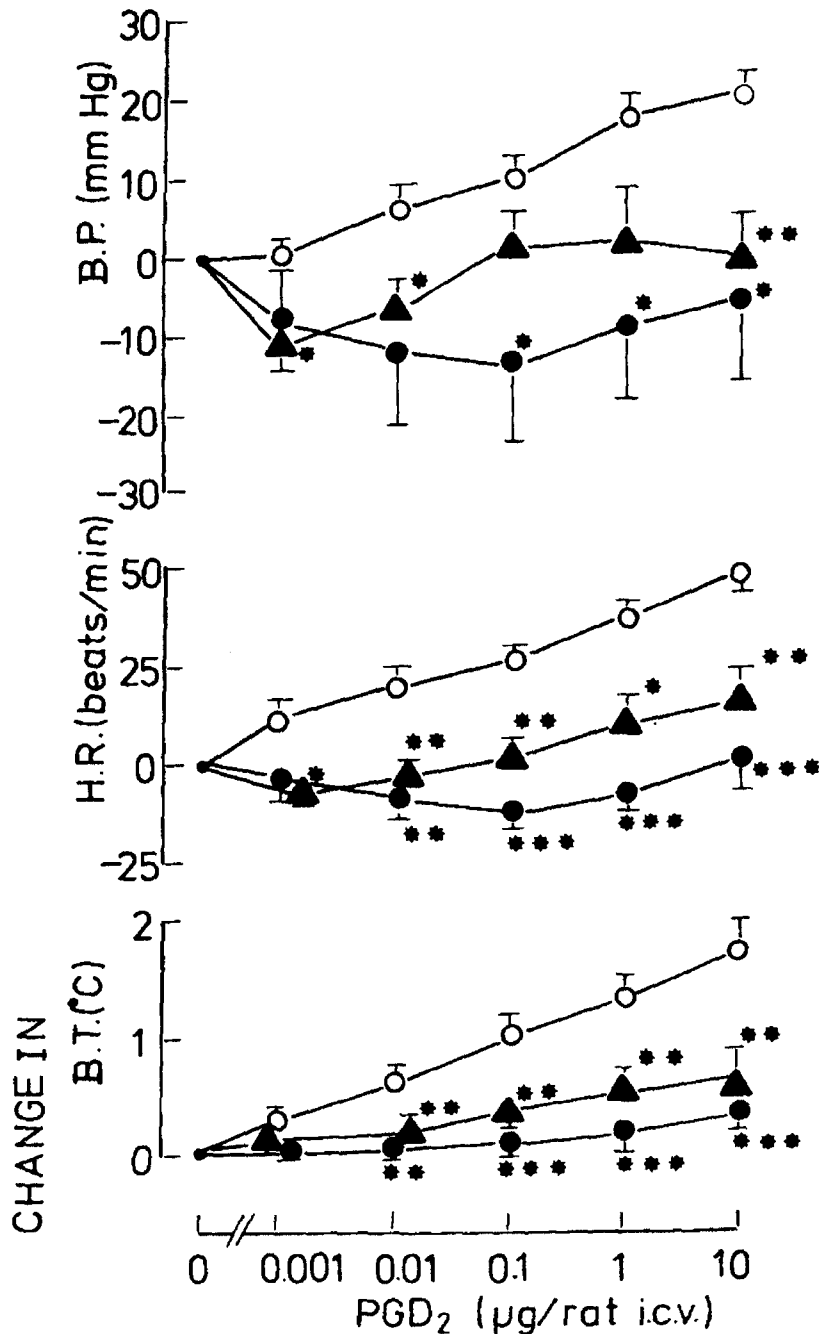


Figure 4. Effect of i.c.v. administered PGD₂ on blood pressure (B.P.), heart rate (H.R.) and body temperature (B.T.) in indomethacin and sodium meclofenamate pretreated rats. Indomethacin, 1 mg/rat (▲—▲), sodium meclofenamate, 1 mg/rat (●—●), or vehicle (○—○) was administered i.c.v. 20 min before commencement of the administrations of PGD₂. Increasing doses of PGD₂ were administered i.c.v. at 20 min intervals. The initial levels for B.P., H.R. and B.T. (means ± s.e.m.) before commencement of the PGD₂ administrations were 103 ± 7 mm Hg, 440 ± 15 beats/min and 35.8 ± 0.2 C in the control group, 100 ± 5 mm Hg, 440 ± 10 beats/min and 35.8 ± 0.3 C in the indomethacin pretreated group, and 90 ± 7 mm Hg, 430 ± 10 beats/min and 35.4 ± 0.3 C in the sodium meclofenamate pretreated group. The differences between the initial levels of the groups are not statistically significant. Vertical bars indicate s.e.m. Each group comprised 6 rats. *p<0.05, **p<0.005 and ***p<0.001 vs. control group.

Effects of prostaglandin D₂ in indomethacin and sodium meclofenamate pretreated rats (Fig. 4)

Central pretreatment with indomethacin (1 mg/rat i.c.v.) effectively antagonised the hypertensive, tachycardic and hyperthermic effects of centrally administered PGD₂.

Pretreatment of the rats with sodium meclofenamate (1 mg/rat i.c.v.) completely blunted the PGD₂-induced central rises of blood pressure, heart rate and body temperature.

DISCUSSION

PGD₂ i.c.v. at the doses of 0.001-10 µg/rat raised the blood pressure of urethane-anaesthetised rats. The highest dose induced an initial decrease in blood pressure, followed by the hypertensive response. I.c.v. administered PGD₂ also dose-dependently increased the heart rate and body temperature of the rats. The effects of PGD₂ were not due to any leakage of the drug into the peripheral circulation but to an action upon the central nervous system, since upon intravenous injections the same doses first decreased and then only slightly increased the blood pressure and had only weak tachycardic and hyperthermic effects. The antagonism of the central cardiovascular and thermal effects of PGD₂ by i.c.v. pretreatments with indomethacin or sodium meclofenamate further supports the assumption of a central site of action for PGD₂.

In agreement with the present results i.c.v. administered PGD₂ at a dose of 10 µg/rat slightly increased the blood pressure and heart rate of the urethane-anaesthetised rat (10). Negligible rise of body temperature has also been reported following i.c.v. injections of PGD₂ in conscious rats (16) and cats (17). The rise of blood pressure after the administration of the smallest dose of PGD₂ preceded the increases in heart rate and body temperature. Hence, the hypertensive response to PGD₂ is not a consequence of the increased body temperature, and PGD₂ seems to be more potent in inducing changes in blood pressure than in heart rate and body temperature.

PGD₂ is the most common prostaglandin type in the rat brain (2). Therefore the formation of PGD₂ in the brain might, under some physiological or pathophysiological conditions, influence cardiovascular and thermoregulatory systems. In fact, the central effects of PGD₂ were very similar to those of arachidonic acid (18,19), the precursor of prostaglandins (14). However, PGD₂ was less potent than PGE₂ (1) or PGF₂α (4, 10) in inducing increases in blood pressure, heart rate and body temperature in the urethane-anaesthetised rat. Therefore, in spite of the abundance of PGD₂ in the rat brain, the weak potency does not suggest an important role for this compound in the central control of the cardiovascular and thermoregulatory systems.

Central pretreatment with indomethacin or sodium meclofenamate effectively antagonised the cardiovascular and thermal responses to i.c.v. administered PGD₂. In agreement with the present findings an antagonism of the effects of exogenous prostaglandins by fenamates and indomethacin

has been reported in several tissues: Sodium meclofenamate and other fenamates antagonise the effects of exogenous prostaglandins in rat, guinea-pig and human alimentary muscle (11,12), human bronchial muscle (20), and these drugs attenuate the $\text{PGF}_{2\alpha}$ -induced bradycardia and hypotension in the cat (21) and prostaglandin-induced gastric acid secretion in the rat (22). Furthermore, the central hypotensive effect of PGI_2 was antagonised by sodium meclofenamate (13). Indomethacin has been reported to blunt the PGD_2 -induced contractions of the longitudinal muscle of rat gastric fundus (12) and to reduce submaximal contractions to PGE_1 in gerbil colon (23). Antagonism of the prostaglandin action by indomethacin has also been demonstrated in rat uterus (24) and in isolated guinea-pig ileum and rabbit ear preparations (25). Indomethacin partly antagonised also the central effects of PGE_2 on blood pressure, heart rate and body temperature of the urethane-anaesthetised rat, while sodium meclofenamate blunted only the hypertensive effect of centrally administered PGE_2 (1). Indomethacin and mefenamic acid both interfere with the $^3\text{H-PGE}_1$ -binding in rat epididymal fat cells suggesting that these agents may directly interact with PGE_1 at a common receptor (23). However, both indomethacin and fenamates effectively inhibit also the synthesis of prostaglandins (14). The total amount of prostaglandins, endogenous plus exogenous, present at the receptors may be decreased by inhibitors of prostaglandin synthesis. Therefore the possibility cannot be excluded that the effects of indomethacin and sodium meclofenamate might be at least partly due to the inhibition of the endogenous prostaglandin synthesis and not solely to the blockade of prostaglandin receptors.

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