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CARDIOVASCULAR RESPONSES INDUCED IN FREE-MOVING RATS BY IMMUNE CYTOKINES

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SUMMARY

1. We investigated the effect of intraperitoneal (I.P.) injections of the immune cytokines, interleukin-1β (IL-1β) and tumour necrosis factor (TNF) on cardiovascular responses in free-moving rats, using a biotelemetry system.

2. The I.P. injection of a small dose of IL-1β (1 μg/kg) induced a monophasic increase in the heart rate, and that of a large dose (10 μg/kg) induced biphasic increases in the blood pressure and heart rate. However, the I.P. injection of any of several doses of TNF (1, 10 and 50 μg/kg) had no effect on cardiovascular responses in rats.

3. Pre-treatment with I.P. injection of indomethacin (10 mg/kg), an inhibitor of cyclo-oxygenase, significantly suppressed the cardiovascular responses and the increase in the plasma noradrenaline (NA) concentration induced by I.P. injection of IL-1β.

4. Microinjection of IL-1β (1 and 10 ng) into the preoptic and anterior hypothalamic (PO–AH) region induced dose-dependent increases in the blood pressure and heart rate in rats. These responses were also suppressed by pre-treatment with I.P. indomethacin (10 mg/kg). In addition, microinjection of prostaglandin E_2 (20 and 100 ng) into the PO–AH region increased blood pressure and heart rate, but that of prostaglandin D_2 (100 ng) had no effect.

5. The present results suggest that IL-1β stimulates the release of prostaglandins, presumably E series, near regions of the hypothalamus, which act on the hypothalamus to induce activation of the sympathetic nervous system. Subsequently, the blood pressure, heart rate and the plasma level of NA increase.

INTRODUCTION

Under infectious or inflammatory conditions, a host reacts with several responses, including fever (Morimoto, Sakata, Watanabe & Murakami, 1989*a*), release of several kinds of hormones (Kasting & Martin, 1982; Morimoto, Murakami,

Nakamori, Sakata & Watanabe, 1989*b*) and metabolic responses known as the acute-phase response (Kampschmidt, 1980; Morimoto *et al.* 1989*a*). These responses are now generally recognized to constitute a part of the host defense reactions to microbial invasion (Kluger, Ringler & Anver, 1975; Kluger & Rothenberg, 1979). Furthermore, increasing evidence has been collected which suggests that these responses are mediated by several kinds of immune cytokines (Dinarello, 1984; Hamilton & Coceani, 1984; Morimoto *et al.* 1989*a*), which are released by circulating and reticuloendothelial leucocytes in response to pathogenic stimuli such as bacterial endotoxin. Among these cytokines, interleukin-1 (IL-1) and tumour necrosis factor (TNF) are well known as potent inducers of fever and metabolic responses (Morimoto *et al.* 1989*a*), as well as immunoregulatory responses.

After systemic injection of bacterial endotoxin, blood pressure and heart rate increase, and fever develops (Skarnes, Brown, Hull & McCraken, 1981; Shido & Nagasaka, 1986). In addition, it has been recently reported that IL-1 stimulates the vascular endothelial cells to produce endothelin which has a potent contractile effect on vascular smooth muscle (Yoshizumi, Kurihara, Morita, Yamashita, Ohhashi, Sugiyama, Takaku, Yanagisawa, Masaki & Yazaki, 1990). Therefore, we have speculated that cytokines may be involved in the development of the cardiovascular responses. In the present study, we examined the effect of systemic injections of IL-1 or TNF on cardiovascular responses (blood pressure and heart rate) in free-moving rats. The present results show that systemic administration of IL-1 significantly increases both blood pressure and heart rate in rats, but TNF has little effect on cardiovascular responses.

It is well known that IL-1 stimulates the structures near the central nervous system (CNS) to synthesize and release prostaglandins of the E series, which act on the hypothalamus to induce fever (Morimoto, Murakami, Nakamori & Watanabe, 1987). In addition, a specific point of action of IL-1 is believed to be in the preoptic and anterior hypothalamic (PO–AH) region, since local injection of IL-1 into this region induced fever, whereas it has little effect on body temperature when injected into the other regions in the CNS (Morimoto, Murakami, Nakamori, Skata & Watanabe, 1989*c*). Therefore, we investigated the effect of microinjection of IL-1, prostaglandin E_2 or prostaglandin D_2 into the PO–AH region on the cardiovascular responses in free-moving rats. Microinjection of IL-1 or prostaglandin E_2 into the PO–AH region increased the blood pressure and heart rate, but that of prostaglandin D_2 had no effect on cardiovascular responses.

In the present study, to determine the possible involvement of prostaglandins in cardiovascular responses induced by IL-1, we further examined the effect of pre-treatment with indomethacin, an inhibitor of prostaglandin synthesis, on changes in the cardiovascular responses and the plasma concentration of noradrenaline (NA) induced by IL-1.

METHODS

Animals

Male albino rats (Wistar strain) weighing 280–360 g were used in this study. The rats were housed in individual plastic cages in a room maintained at 26 ± 1 °C, a temperature within the thermoneutral zone for rats, with 12:12 h light–dark cycle, with light on 07.00 h. Tap water and rodent chow were provided *ad libitum*.

Measurement of physiological parameters

Blood pressure, heart rate and physical activity were measured using a biotelemetry system (DATAQUEST III, Data Science Inc. USA) (Lange, Brockway & Azar, 1991). Under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.), a battery-operated telemetric transmitter was implanted intraperitoneally at least 10 days before the start of the experiment. During the surgery, the tip of the cannula to measure the blood pressure was inserted into the descending aorta. Output (frequency in Hz) from the transmitter was monitored by antennae mounted in a receiver board that was placed under each animal's cage (length 40 cm, width 25 cm, depth 25 cm). The data were fed into a peripheral processor connected to a microcomputer (Sanyo). The rat's activity was measured using the same transmitter and the same biotelemetry system described above. In this system, changes in activity are detected by changes in the position of the transmitter with respect to the antennae mounted in the board. Changes in the position of the transmitter alter the strength of the signal detected by the antennae. Each change in signal strength is recorded as a 'pulse' of activity.

Microinjection into the PO–AH region

For microinjection into the PO–AH region, five rats in a separate group had been implanted previously with a stainless-steel guide cannula (0·8 mm o.d.) at co-ordinates AP 2·0, L 1·0, V 8·5 mm according to the rat brain atlas (Pellegrino, Pellegrino & Cushman, 1979) by standard stereotaxic techniques. The implant assembly was fixed to the skull with dental acrylic cement and two stainless-steel screws. This implantation was done under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.) at least 14 days before the surgery of I.P. implantation of the telemetric transmitter. Intra-PO–AH injections were made through a sterile stainless-steel needle (0·4 mm o.d.) attached to a polyethylene tube; the volume infused was always 1 μl. Each microinjection was performed over a period of about 1 min. After completion of experiments, the animals were killed by a large dose of pentobarbitone I.P. The positions of the tips of the guide cannulae were histologically identified.

Measurement of plasma NA

To measure the plasma concentration of NA, a group of rats ($n = 8$) without transmitters was used. For blood sampling, the eight animals had been previously catheterized with a cannula under general anaesthesia (sodium pentobarbitone, 50 mg/kg, I.P.). Polyvinyl tubing was inserted into the jugular vein, such that the tip of the tubing was located in the superior caval vein near the right atrium. The free end of the catheter was passed subcutaneously to the mid-scapular region, where it was exteriorized dorsally, behind the neck. It was kept patent by flushing it every day with heparinized 0·9% saline (50 U/ml). This implantation was performed at least 3 days before the blood sampling. For measuring the plasma concentration of NA, about 0·5 ml of blood was withdrawn through the cannula. The blood samples were taken twice, 90 min before and 180 min after I.P. injection of IL-1β. Blood was collected into test-tubes containing EDTA and was centrifuged at 2000 r.p.m. for 15 min at 4 °C. Then the plasma was transferred into new test-tubes and stored at −40 °C until the measurement of NA was performed. The plasma NA concentration was measured by a standard method using HPLC (high-pressure liquid chromatography).

Interleukin-1, tumour necrosis factor, prostaglandins and indomethacin

Human recombinant interleukin-1β (IL-1β) and human recombinant tumour necrosis factor (TNF) were supplied by, respectively, Otsuka Pharmaceutical Co. Ltd, Japan and by Dainippon Pharmaceutical Co. Ltd, Japan. The IL-1β and TNF had been produced by recombinant strains of *Escherichia coli*. They were carefully produced and were endotoxin-free as confirmed by a *Limulus* amoebocyte lysate test ($< 0{\cdot}05$ pg/μg protein). The biological activity of IL-1β which was assayed by thymocyte co-stimulation activity was 2×10^7 U/mg protein. The activity of the TNF, which was based on the cytotoxic activity against L-M cells, was $3{\cdot}2 \times 10^6$ U/mg protein. The molecular weight of IL-1β was 17400 and that of TNF was 17000. For injection, IL-1β and TNF were dissolved in sterile saline at a concentration of 1 or 10 μg/ml. These solutions were divided into several vials and stored at −40 °C until use. We used each vial within 4 days after thawing and have avoided repeat freezing and thawing. Prostaglandins E_2 and D_2 were dissolved in sterile saline containing 0·5% ethanol at concentrations of 20 or 100 μg/ml. We used saline containing

0·5% ethanol as the control vehicle for the prostaglandin solution. To minimize any confounding effects of the circadian rhythm for physiological parameters, intraperitoneal or intra-PO–AH injections of cytokines or prostaglandins were always made between 12.00–13.00 h. Indomethacin was dissolved in saline containing 4% sodium bicarbonate at a concentration of 10 mg/ml. We used saline containing 4% sodium bicarbonate as the control vehicle for the indomethacin solution.

The data were analysed for statistical significances by Student's t test.

RESULTS

Figure 1 shows the blood pressure (A), heart rate (B) and physical activity (C) of a representative rat over a period of 5 days. The shaded bars at the top of the figure indicate the periods of darkness. Each parameter was measured at 1 min intervals, and each data point represents the average value over 15 min. Note that all three physiological parameters show typical circadian rhythmicity. The blood pressure, heart rate and activity increase during dark periods and decrease during the light periods. On day 2, the rat received an I.P. injection of a small dose of IL-1β (1 μg/kg). This resulted in a slight increase in blood pressure, heart rate and activity but these

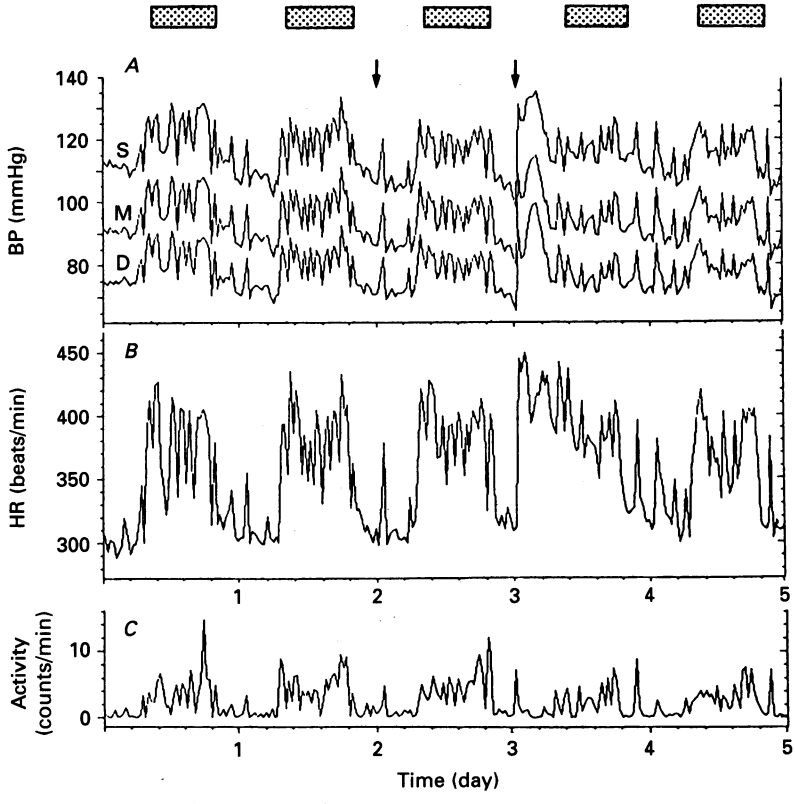

Fig. 1. Changes in blood pressure (BP, A), heart rate (HR, B) and activity (C) of a rat. Shaded bars at the top indicate dark periods. Arrows indicate the injection time of interleukin-1β (1 μg/kg on day 2, 10 μg/kg on day 3). S, systolic pressure; M, mean arterial pressure; D, diastolic pressure.

values soon returned to their initial levels. However, the I.P. injection of a larger dose of IL-1β (10 μg/kg) on day 3 caused a marked increase in blood pressure and heart rate that lasted more than 7 h. In contrast to the prolonged changes in blood pressure and heart rate, the activity of the rat following the I.P. injection of IL-1β showed

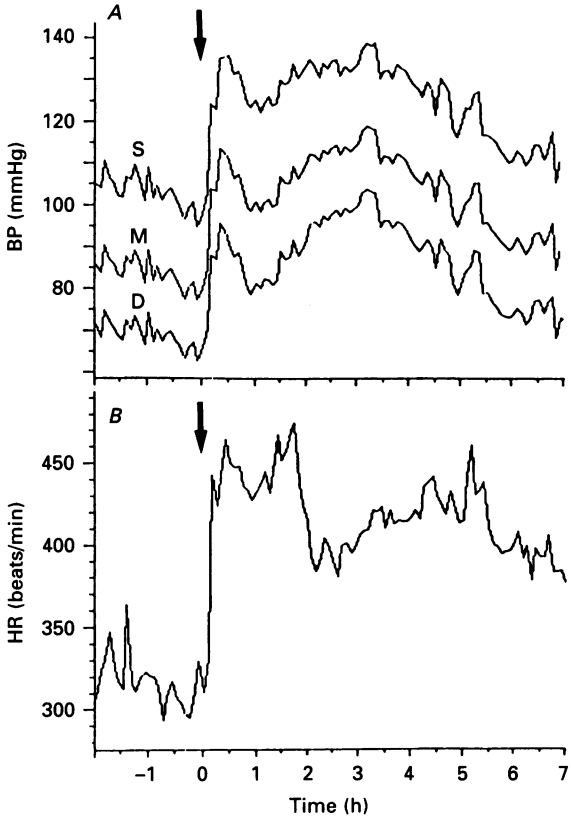

Fig. 2. Changes in blood pressure (BP, *A*), and heart rate (HR, *B*) after I.P. injection of interleukin-1β (10 μg/kg). Arrows indicate the injection time. S, systolic pressure; M, mean arterial pressure; D, diastolic pressure.

only a transient elevation, indicating that the cardiovascular responses were not the result of a change in the physical activity of the rat.

Figure 2 shows the changes in blood pressure and heart rate after the I.P. injection of the higher dose of IL-1β (10 μg/kg). These are the same data as presented in Fig. 1*A* and *B* (day 3), but are plotted at 5 min intervals. The blood pressure response showed a biphasic increase. The first peak occurred about 30 min after injection of IL-1β, and the second peak at about 3 h. It should also be noted that the changes in systolic and diastolic pressures occurred almost in parallel. The pattern of response of heart rate was also biphasic but the time to each peak did not always correspond with that observed in the blood pressure response.

Figure 3 shows changes in the mean arterial blood pressure of six rats after the I.P. injection of IL-1β (Fig. 3*A*), and TNF or saline (Fig. 3*B*). In Fig. 3*A*, the I.P. injection of a high dose of IL-1β (10 μg/kg) induces biphasic responses in the blood pressure. The first peak occurred at 30 min and the second peak at 3·5 h. The

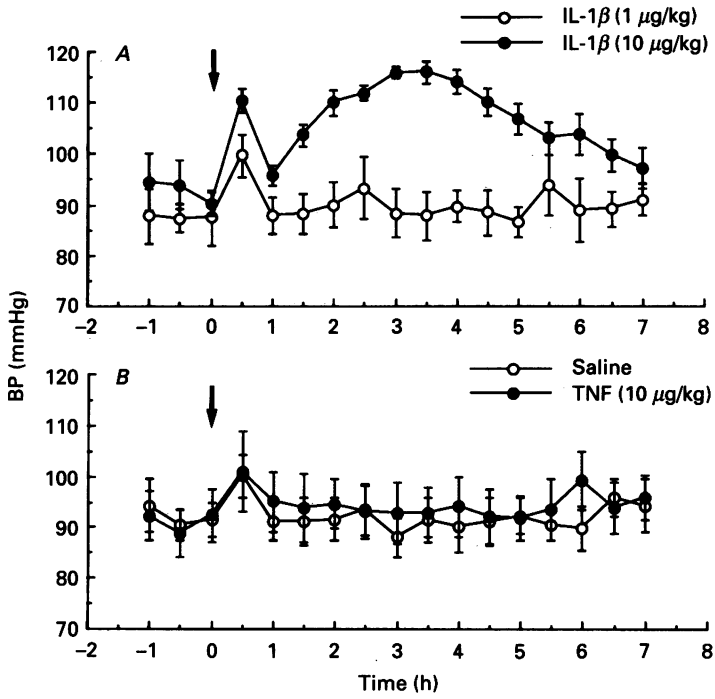

Fig. 3. Changes (mean ± S.E.M.) in the mean arterial blood pressure (BP) of the same group of six rats after injections of interleukin-1β (IL-1β, *A*), and tumour necrosis factor (TNF) or saline (*B*). Arrows indicate the injection time.

magnitude of the second peak (116 ± 2·2 mmHg) was significantly ($P < 0·05$) greater than that of the first peak (110·5 ± 2·2 mmHg). The lower dose of IL-1β (1 μg/kg) induced a monophasic response. However, this monophasic response was not significant as compared with the response induced by I.P. injection of saline (Fig. 3*B*). As shown in Fig. 3*B*, however, no significant changes in the blood pressure were observed after I.P. injections of TNF (10 μg/kg) or saline (1 ml/kg).

Figure 4 shows changes in the heart rate after the I.P. injection of IL-1β (Fig. 4*A*), TNF or saline (Fig. 4*B*). In Fig. 4*A*, I.P. injections of the higher dose of IL-1β (10 μg/kg) induced biphasic responses in the heart rate and those of a small dose (1 μg/kg) induced a monophasic response. The first peak occurred at 30 min, corresponding to the first peak of the blood pressure (Fig. 3*A*). However, the second peak occurred at 4–5 h post-injection, which was different from the time of the second peak in blood pressure. As shown in Fig. 4*B*, however, no significant changes in heart rate are observed after I.P. injections of TNF (10 μg/kg). In addition, we examined the effect of I.P. injection of 1 or 50 μg/kg of TNF on cardiovascular responses in free-moving rats, but those doses also had no effect.

Figure 5 shows the effect of pre-treatment with I.P. injection of indomethacin (10 mg/kg) on changes in the mean arterial blood pressure (Fig. 5*A*) and heart rate (Fig. 5*B*) induced by I.P. injection of IL-1β (10 μg/kg). Indomethacin or vehicle was injected 90 min before injection of IL-1β. The baseline values at the time zero of the

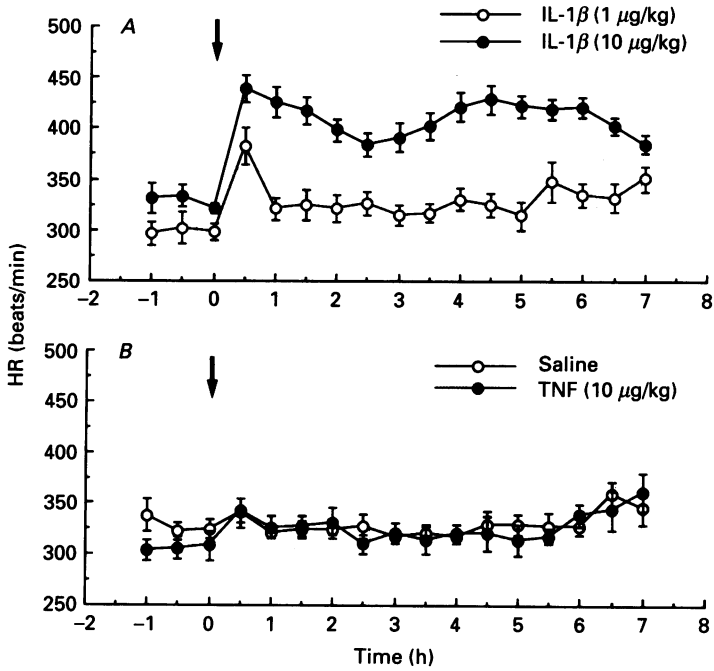

Fig. 4. Changes (mean ± S.E.M.) in the heart rate of the same group of six rats after I.P. injections of interleukin-1β (IL-1β, *A*), tumour necrosis factor (TNF) or saline (*B*). Arrows indicate the injection time.

blood pressure and heart rate were 105·5 ± 14·5 mmHg and 317·1 ± 21·7 beats/min in the indomethacin-injected group, and 93·3 ± 7·4 mmHg and 314·5 ± 17·1 beats/min in the vehicle-injected group. There were no significant differences between these values. As shown in Fig. 5*A*, the increases in the blood pressure were significantly ($P < 0{\cdot}001$) suppressed by pre-treatment with I.P. indomethacin. The increases in heart rate were also significantly ($P < 0{\cdot}02$) suppressed by this treatment. In addition, Fig. 6 shows that the plasma concentration of noradrenaline (NA) significantly increases 3 h after I.P. injection of IL-1β. This increase in the plasma NA concentration was also suppressed by the pre-treatment with I.P. injection of indomethacin.

Figure 7 shows changes in the mean arterial blood pressure (*A*) and heart rate (*B*) of five rats after microinjection of IL-1β or saline into the PO–AH region. Microinjection of IL-1β (1 and 10 ng) into the PO–AH region induced biphasic increases in the blood pressures and heart rate in a dose-dependent manner. The first peak occurred at 30 min and the second peak at 1·5 h. As shown in Fig. 7, however, no significant changes are observed after intra-PO–AH injection of saline (1 μl).

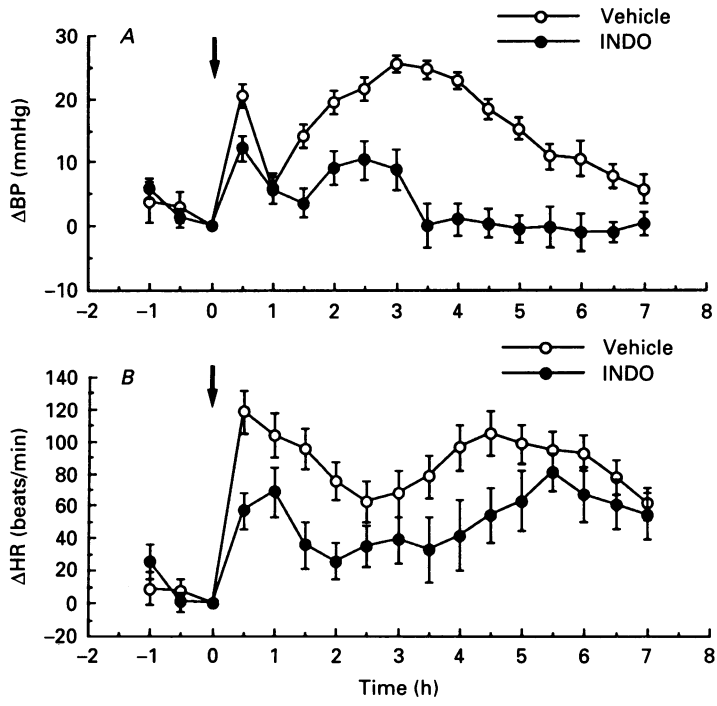

Fig. 5. Effect of pre-treatment with the I.P. injection of indomethacin (INDO) or vehicle on changes in the mean arterial blood pressure (ΔBP, *A*) and heart rate (ΔHR, *B*) of the same group of six rats after the I.P. injection of interleukin-1β (10 μg/kg). Arrows indicate the time of interleukin-1β injection. Indomethacin or vehicle was injected 90 min before the injection of interleukin-1β.

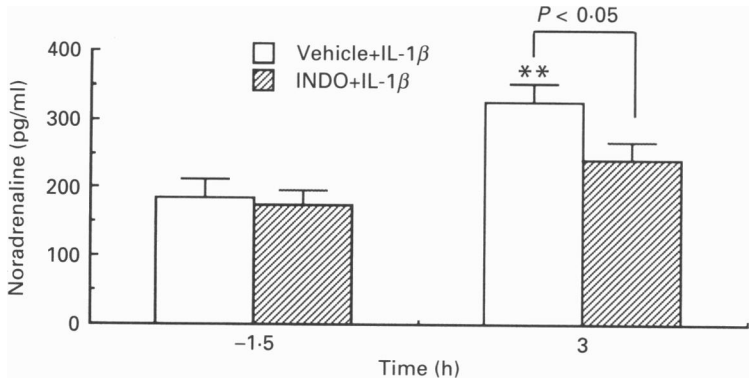

Fig. 6. Effect of pre-treatment with I.P. injection of indomethacin (INDO) or vehicle on changes in the plasma concentration of noradrenaline in rats ($n = 8$) after the I.P. injection of interleukin-1β (10 μg/kg).

Figure 8 shows the effect of pre-treatment with I.P. injection of indomethacin (10 mg/kg) on the cardiovascular responses induced by intra-PO–AH injection of IL-1β (10 ng). Indomethacin or vehicle was injected 90 min before intra-PO–AH

injection of IL-1β. As shown in Fig. 8*A*, the increases in the blood pressure were significantly ($P < 0{\cdot}01$) suppressed by pre-treatment with I.P. indomethacin. The increases in heart rate were also significantly ($P < 0{\cdot}05$) suppressed by this treatment.

Figure 9 shows changes in the mean arterial blood pressure (*A*) and heart rate (*B*) of five rats after microinjection of prostaglandin E_2 or D_2 into the PO–AH region.

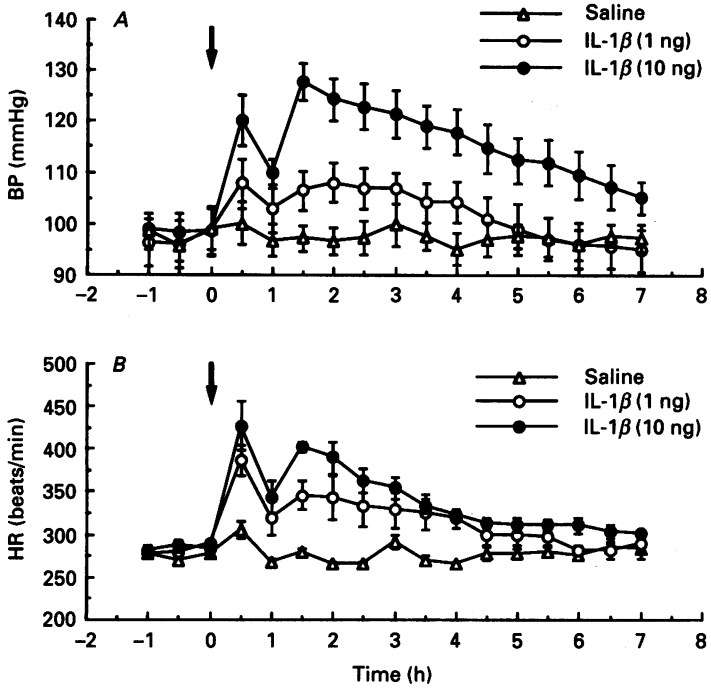

Fig. 7. Changes (mean ± S.E.M.) in the mean arterial blood pressure (*A*) and heart rate (*B*) of a group of five rats after microinjection of interleukin-1β (IL-1β) or saline into the PO–AH region. Arrows indicate the injection time.

Microinjection of prostaglandin E_2 (20 and 100 ng) increased the blood pressure and heart rate in a dose-dependent manner. However, intra-PO–AH injection of prostaglandin D_2 (100 ng) had little effect on cardiovascular responses. No significant changes were observed between the cardiovascular responses induced by prostaglandin D_2 and those produced by saline. Furthermore, pre-treatment with I.P. indomethacin (10 mg/kg) had no effect on cardiovascular responses induced by intra-PO–AH injection of prostaglandin E_2 (not illustrated).

DISCUSSION

The present results show that microinjections of either a small (1 ng) or a large (10 ng) dose of IL-1β into the PO–AH region induce biphasic increases in blood pressure and heart rate in free-moving rats. However, I.P. injections of a small dose (1 μg/kg) of IL-1β caused a monophasic increase in the blood pressure and heart rate whilst a large dose (10 μg/kg) produced biphasic responses. This may indicate that

a dose of 1 μg/kg of IL-1β injected I.P. is not enough to activate the CNS to induce the biphasic responses. Comparing the time course of the biphasic responses of the blood pressure and heart rate, the latency to the first peak induced by intra-PO–AH injection was almost the same as that induced by I.P. injection. However, the latency

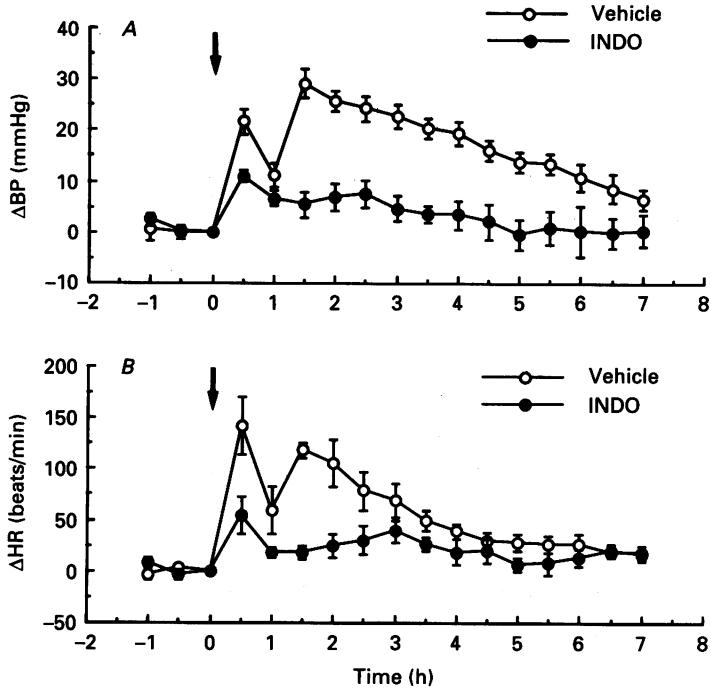

Fig. 8. Effect of pre-treatment with the I.P. injection of indomethacin (INDO) or vehicle on changes in the mean arterial blood pressure (ΔBP, *A*) and heart rate (ΔHR, *B*) of a group of five rats after microinjection of interleukin-1β (10 μg/kg). Arrows indicate the time of interleukin-1β injection. Indomethacin or vehicle was injected 90 min before the microinjection of interleukin-1β.

to the second peak induced by intra-PO–AH injection was shorter than that by I.P. injection.

Recently, Morimoto *et al.* (1989*a*) have reported that a small dose of IL-1 causes a monophasic increase in the body temperature (fever) and a large dose produces biphasic fever when IL-1 is intravenously injected into rabbits. The present results show that the time course of the biphasic rises in blood pressure after an I.P. injection of IL-1β corresponds with that the biphasic febrile response of rats (Morimoto, Watanabe, Ono, Sakata & Murakami, 1986). Therefore we speculated that similar mechanisms are involved in the cardiovascular and febrile responses induced by IL-1.

It has been believed that IL-1 causes fever and hormonal responses by its direct action on the hypothalamus. However, since cytokines such as IL-1 are proteins with molecular weights of 15000–20000 (Dinarello, 1984), their passage through the blood–brain barrier is likely to be limited. The organum vasculosum laminae

terminalis (OVLT), located in the anterior wall of the third ventricle is one of the circumventricular organs where the blood–brain barrier is relatively loose, and is currently thought to be the pathway by which IL-1 enters the PO–AH region and/or the site of prostaglandin synthesis in response to IL-1 (Blatteis, Bealer, Hunter,

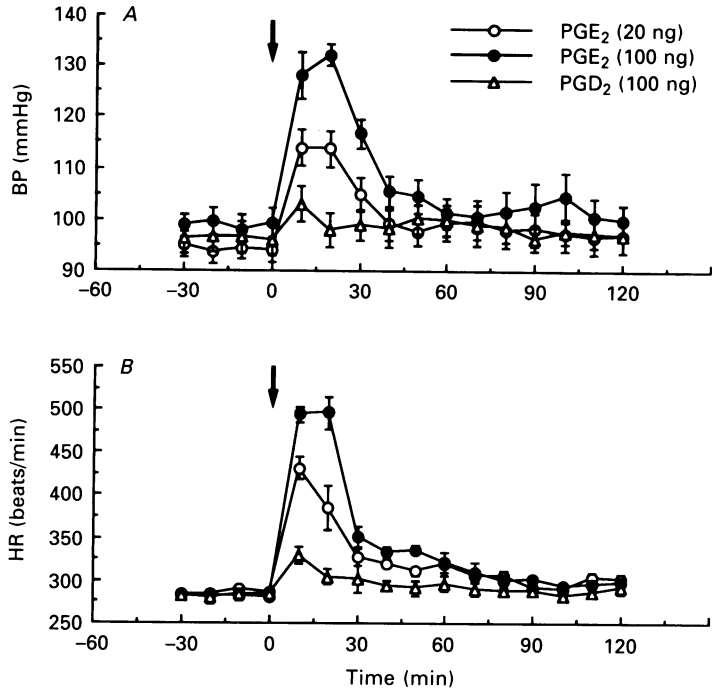

Fig. 9. Changes (mean ± S.E.M.) in the mean arterial blood pressure (*A*) and heart rate (*B*) of a group of five rats after microinjection of prostaglandin E_2 (PGE_2) or prostaglandin D_2 (PGD_2) into the PO–AH region. Arrows indicate the injection time.

Llanos, Ahokas & Mashburn, 1983). In the present study, therefore, we examined the effect of microinjection of IL-1β into the PO–AH region located near the OVLT on cardiovascular responses.

Since prostaglandins of the E series have been shown to be highly pyrogenic when injected into the cerebral ventricle (Milton & Wendandt, 1970) or the hypothalamus (Stitt, 1973), it has been generally believed that prostaglandins acting in the CNS are the final mediators of the febrile response. In addition, it has been recently shown that prostaglandin synthesis involved in the febrile response induced by IL-1 occurs both inside and outside the blood–brain barrier (Morimoto *et al.* 1987). This hypothesis was further substantiated by the fact that prostaglandin E levels increase in both the blood circulation (Skarnes *et al.* 1981; Rotondo, Abul, Milton & Davidson, 1988) and the cerebrospinal fluid during fever (Feldberg & Gupta 1973; Bernheim, Gilbert & Stitt, 1980). Moreover, prostaglandins of the E series injected into the cerebral ventricle (Malkinson, Cooper & Veale, 1990) or the hypothalamus (Feuerstein, Adelberg, Kopin & Jacobowitz, 1982) also affect the cardiovascular system by causing increases in blood pressure and heart rate.

In the present results, increases in blood pressure and heart rate induced by either I.P. or intra-PO–AH injection of IL-1β were significantly suppressed by pre-treatment with indomethacin. The increase in the plasma NA level after I.P. injection of IL-1β was also significantly suppressed by pre-treatment with indomethacin. As it is well known that prostaglandin E_2 and D_2 are synthesized in the CNS, we examined the effect of microinjection of prostaglandin E_2 or D_2 on cardiovascular responses. Intra-PO–AH injection of prostaglandin E_2 induced marked increases in blood pressure and heart rate but prostaglandin D_2 had no effect. Since prostaglandin E_2 receptors are found exclusively in the PO–AH region (Matsumura, Watanabe, Onoe, Watanabe & Hayaishi, 1990), we speculate that IL-1β in the PO–AH region stimulates the synthesis and release of prostaglandin E_2, which acts on the neurones in that region to cause the sympathetic nervous activation. Subsequently, blood pressure and heart rate increase. This is supported by the fact that intrahypothalamic injection of prostaglandin E_2 causes an elevation in the plasma concentrations of catecholamines (Feuerstein *et al.* 1982).

Prostaglandins released into the circulation may also be involved in cardiovascular responses. It has been reported that intracarotid infusions of prostaglandin E_2 increase blood pressure and heart rate in conscious sheep (Skarnes *et al.* 1981; Breuhaus, Demarest & Chinoskey, 1989). About 90% of all prostaglandins in the circulation are metabolized during each pulmonary circulation (Piper, Vane & Wyllie, 1970). However, Egucshi, Hayashi, Urade, Ito & Hayaishi (1988) reported that 0·13% of the administered dose of prostaglandin E_2 was transported into the rat brain, when prostaglandin E_2 was intravenously injected at a concentration of 1 mg/kg. Therefore, it is possible that the increased concentration of prostaglandin E_2 in the circulation after a systemic injection of IL-1 (Rotondo *et al.* 1988) contributes to the enhancement of cardiovascular responses.

TNF is also well known as a pyrogenic cytokine (Morimoto *et al.* 1989*a*). However, in our experiments, the I.P. injection of TNF had no effect on cardiovascular responses in free-moving rats. This result may be explained by the fact that the ability of TNF to induce physiological responses is less than one-tenth of that of IL-1 (Morimoto *et al.* 1989*a*).

Recently Beasley, Cohen & Levinsky (1989) have reported that exposure of isolated rat's aortic rings to IL-1 for 1 h did not affect phenylephrine-induced contractions, but contractions were markedly decreased 150–200 min after the initiation of IL-1 exposure. They concluded that IL-1 is a potent inhibitor of vascular contraction. Their explanation may be tenable. However, it is known that clearance of IL-1 from the blood circulation is quite rapid (within 10 min; Dinarello, 1984). Therefore the effect observed *in vitro* does not necessarily seem to appear *in vivo*. However, during severe infection such as sepsis, a large concentration of IL-1 may be continuously released into the blood circulation. Systemic vasodilatation occurs during septic shock or after injection of very high doses of bacterial endotoxin and, consequently hypotension is induced (Mathison, Wolfson & Ulevitch, 1988). Therefore it may be possible that, as Weinberg, Wright & Guz (1988) reported, a high concentration of IL-1 remaining in the blood circulation for a long period of time may cause hypotension. TNF may also be involved in hypotension induced by administration of a large dose of endotoxin (Mathison *et al.* 1988).

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