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# Effect of indomethacin on the blood pressure and plasma catecholamine responses to acute endotoxaemia

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Injection of *E. coli* endotoxin (7 mg $kg^{-1}$ i.v.) to pentobarbitone anaesthetized cats resulted in prolonged decrease of systemic blood pressure and increases in plasma concentrations of adrenaline, noradrenaline and 6-ketoprostaglandin (PG) $F_{1\alpha}$. Indomethacin pretreatment (10 mg $kg^{-1}$ i.v.) attenuated the decrease in blood pressure following endotoxin injection. Plasma adrenaline, noradrenaline and 6-keto-PG $F_{1\alpha}$ were significantly lower in the indomethacin-treated cats. These data indicate that the superior haemodynamic status of the indomethacin pretreated animals exposed to endotoxic shock is not the result of potentiation of the peripheral sympathetic response.

Non-steriodal anti-inflammatory drugs (NSAID) were shown to attenuate the fall in blood pressure and cardiac output following acute endotoxaemia (Hinshaw et al 1967; Parratt & Sturgess 1975; Fletcher & Ramwell 1977). These effects of NSAID were attributed to inhibition of arachidonate metabolism, since plasma levels of various prostaglandins (PG) are markedly elevated during endotoxin shock (Collier et al 1973; Anderson et al 1975; Bult et al 1980). However, prostaglandins are also implicated in the regulation of sympathetic tone by presynaptic inhibition of the release of neuronal noradrenaline (Hedqvist 1977). Thus, it might be possible that the beneficial haemodynamic effects of NSAID in endotoxic shock are the results of potentiation of adrenergic responses due to elimination of the negative modulatory effect of prostaglandins on noradrenaline release from sympathetic nerve endings. The following experiments were designed to examine this hypothesis by following blood pressure and plasma catecholamines in cats exposed to acute *Escherichia coli* endotoxaemia with or without pretreatment with indomethacin, a potent prostaglandin synthesis inhibitor (Vane 1971).

## MATERIALS AND METHODS

Cats of either sex, 2·5–4·0 kg, were anaesthetized with pentobarbitone sodium (45 mg $kg^{-1}$ i.m.) and the left femoral artery and vein were cannulated. The cats were divided in two groups: 1. Control group, which had received infusion of 10 ml of 0·2 M $Na_2CO_3$ (pH adjusted to 7·8) over 60 min, followed by infusion of endotoxin (*E. coli* lipopolysaccharide 055:B5, Sigma, St. Louis, MO) 7 mg $kg^{-1}$, in 10 ml of 0·9% NaCl (saline), over 10 min 2. Indomethacin-treated group which received infusion of 10 mg $kg^{-1}$ of indomethacin (Sigma, St. Louis, MO) dissolved in 0·2 M $Na_2CO_3$, over 60 min, followed by endotoxin injection as described for the control group. Mean arterial blood pressure was continuously recorded by a Grass model 5 polygraph and type 4-327-c pressure transducer. Arterial blood samples (1·0 ml) were withdrawn at the control period (5 min before endotoxin injection), and every 20 min after the endotoxin injection, throughout the experiment (120 min). The blood samples were kept in ice until the end of the experiment and then centrifuged (5000 *g*, 10 min 4 °C), and the plasma preserved at −20 °C. Plasma noradrenaline and adrenaline concentrations were assayed in all the samples. Plasma 6-keto-PG $F_{1\alpha}$ was assayed in the control period and 20, 60 and 120 min after the endotoxin injection. The 6-keto-PG $F_{1\alpha}$, a major metabolite of prostacyclin was assayed directly from plasma (100 $\mu$l) by radioimmunoassay kit (New England Nuclear, Boston, Mass).

Adrenaline and noradrenaline content in 200 $\mu$l of plasma sample were assayed by a radioenzymatic, thin-layer chromatographic procedure, as described by Weise & Kopin (1976). Briefly, the procedure was as follows: the protein-free samples were incubated with catechol-*O*-methyl transferase and tritiated *S*-adenosyl methionine. After incubation, the reaction

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was stopped by addition of borate buffer (pH 8·0) containing authentic metanephrine and normetanephrine. The amines were extracted into toluene–isoamyl alcohol (3 : 2) and then into 0·1 M acetic acid. The radioactive products were separated by thin layer chromatography, and the appropriate areas separately scraped into counting vials. After periodate oxidation of the *O*-methylated compounds to vanillin, phosphor-containing toluene was added and tritium assayed by liquid scintillation chromatography.

The results in text and figures are mean ± s.e.m. Student's *t*-test (two tails) was used for statistical evaluation.

## RESULTS

Fig. 1 shows that injection of *E. coli* endotoxin results in marked decrease of the systemic blood pressure. The mean blood pressure of the control (endotoxin only) group which was 118 ± 4 mmHg before endotoxin injection and decreased by 41 ± 5 mmHg at 40 min after endotoxin injection, with no significant recovery thereafter. The indomethacin treated group, in which the mean arterial blood pressure before endotoxin injection was 114 ± 4 mmHg, showed an initial decrease in blood pressure of only 20 mmHg and a complete recovery to control level at the end of the experiment.

The hypotension induced by endotoxin in the control group was accompanied by increases of plasma concentrations of adrenaline, noradrenaline and 6-keto-PG $F_{1\alpha}$. Plasma concentrations of the 6-keto-PG $F_{1\alpha}$ increased 5 fold: from 0·34 ± 0·12 ng ml$^{-1}$ at the control period, to 1·82 ± 0·45 ng ml$^{-1}$ 120 min after the endotoxin injection (Table 1). Plasma adrenaline (Fig. 2) increased from 42 ± 9

Table 1. Effect of indomethacin (10 mg kg$^{-1}$ i.v.) on plasma concentrations of 6-keto-PG $F_{1\alpha}$ in endotoxin (7 mg kg$^{-1}$) treated cats. n = number of animals in each group. * denote level of significant difference (unpaired *t*-test), between the groups, as follows: * $P < 0.05$; ** $P < 0.01$.

| | Plasma 6-keto-PG $F_{1\alpha}$ (ng ml$^{-1}$) | | | |
|---|---|---|---|---|
| | Control period | Min after endotoxin injection 20 | 60 | 120 |
| Endotoxin (n = 9) | 0·34 ± 0·08* | 0·60 ± 0·18 | 1·20 ± 0·41* | 1·82 ± 0·45** |
| Endotoxin + indomethacin (n = 6) | 0·11 ± 0·05 | 0·35 ± 0·17 | 0·26 ± 0·12 | 0·18 ± 0·05 |

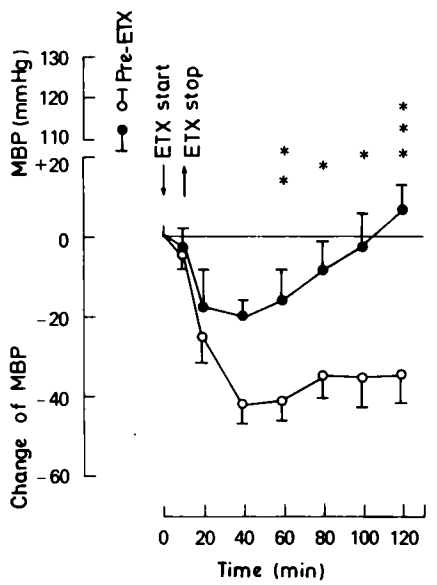


FIG. 1. Effect of indomethacin on blood pressure response to *E. coli* endotoxaemia. ○—○ Control, endotoxin (7 mg kg$^{-1}$ n = 10); ●—● Indomethacin (10 mg kg$^{-1}$ n = 6)—pretreated cats which received *E. coli* endotoxin (7 mg kg$^{-1}$). 0 time denotes the start of the endotoxin infusion, for 10 min. ETX-denotes endotoxin; pre-ETX denotes the mean arterial blood pressure immediately before ETX infusion. * denote level of significant difference (unpaired *t*-test) between the groups, as follows: * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$.

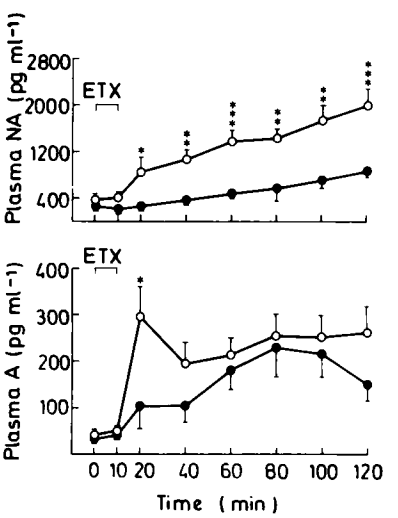


FIG. 2. Effect of indomethacin on plasma adrenaline and noradrenaline concentrations of cats exposed to *E. coli* endotoxaemia. ○—○ Control, endotoxin (7 mg kg$^{-1}$, n = 10). ●—● Indomethacin (10 mg kg$^{-1}$, n = 6) pretreated cats which received *E. coli* endotoxin (7 mg kg$^{-1}$). ETX-denotes period of endotoxin infusion. * denotes level of significant difference (unpaired *t*-test) between groups, as follows: * $P < 0.05$ ** $P < 0.02$ *** $P < 0.01$.

pg ml$^{-1}$ (control period) to 295 ± 87 pg ml$^{-1}$, 20 min after endotoxin injection; thereafter a decrease of plasma adrenaline was followed by a second increase, to 259 ± 66 pg ml at the end of the experiment. Plasma noradrenaline (Fig. 2) gradually increased from 339 ± 37 pg ml$^{-1}$ at the control period, to 1988 ± 276 pg ml$^{-1}$ at the end of the experiment.

In indomethacin pre-treated cats, there was a substantial decrease of plasma 6-keto-prostaglandin $F_{1\alpha}$: the control level of 6-keto-prostaglandin $F_{1\alpha}$ in this group was 0·11 ± 0·07 ng ml$^{-1}$, indicating a significant inhibition of prostaglandin synthesis. Following endotoxin injection to these cats, plasma concentrations of 6-keto-PG $F_{1\alpha}$ showed a transient increase at 20 min after endotoxin infusion which then declined to control values (Table 1).

Plasma adrenaline concentrations of the indomethacin-treated cats were similar to the values observed in the control group except for a short period at 20 min after the endotoxin injection (Fig. 2). The increases in plasma noradrenaline, concentrations, however, were significantly lower in the indomethacin-treated cats, throughout the duration of the endotoxaemia period (Fig. 2).

## DISCUSSION

The data presented in this study demonstrate that pretreatment with indomethacin attenuates the fall in blood pressure which attends endotoxin injection. These results corroborate previous studies in several species (Hinshaw et al 1967; Fletcher & Ramwell 1977; Parratt & Sturgess 1975). The increase in the plasma content of 6-keto-PG $F_{1\alpha}$ during acute endotoxaemia is also in accord with previous observations (Bult et al 1980).

Plasma concentrations of adrenaline and noradrenaline are known to increase during endotoxaemia. However, in previous studies in which attempts were made to evaluate the sympathetic response in endotoxaemia, bioassay or fluorimetric methods were used. The sensitive radioenzymatic method used in the current investigation provides more accurate data for the sympathetic response in this type of shock.

The resting plasma adrenaline concentrations we found are significantly lower than those observed by Spink et al (1966), Nykiel & Glaviano (1961), Hall & Hodge (1971). After endotoxin injection to the control cats, there was a rapid increase of plasma adrenaline concentration which was then sustained at a high value through the rest of the experiment. Plasma noradrenaline showed a different pattern of response: a gradual increase up to a maximum at the end of the experiment. In indomethacin-treated cats, plasma concentrations of both amines (especially noradrenaline) were significantly lower than the values observed in the control cats which received endotoxin only. The lower plasma concentrations of the catecholamines in the indomethacin-treated cats may thus reflect the superior haemodynamic status of these animals, compared with the control group. It is evident that the higher blood pressure observed in the indomethacin-treated cats is not the result of potentiation of the sympathetic response, as might be anticipated according to Hedqvist theory (Hedqvist 1977). Furthermore, the sympathetic nervous system was assumed to be detrimental in the pathogenesis of the endotoxin syndrome since 6-OH dopamine pretreatment retards the development of lethal toxicity after *E. coli* endotoxin injection (Bolton & Atuk 1978). Recently, indomethacin was found to inhibit release of histamine as well as other vasoactive substances through interference with calcium fluxes (Northover 1977). Thus, the lower plasma catecholamine concentrate observed in indomethacin-pretreated cats may not be merely the result of the superior haemodynamic situation, and therefore a lesser sympathetic stimulation, but may represent a more direct effect of indomethacin on catecholamine release through interference in the release process. This possibility is further supported by experiments conducted in our laboratory (unpublished observation) in which comparable doses of indomethacin (10–15 mg kg$^{-1}$) suppressed the release of noradrenaline and adrenaline induced by spinal cord stimulation in the pithed rat. However, further investigations are needed to elucidate the effect of indomethacin on adrenergic neurotransmitter release in intact animals.

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