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Dexamethasone fails to produce antipyretic and analgesic actions in experimental animals

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Abstract

In order to explore the role of phospholipase A_2 inhibition in the mechanisms of the action of glucocorticoids, it was investigated whether the steroid exhibits the analgesic and antipyretic actions as well as cyclo-oxygenase inhibitors such as indomethacin or not. Dexamethasone has been reported to produce the anti-inflammatory action with a lag time of at least 1 h at doses of up to 0.1 mg/kg in mice and rats. However, dexamethasone when given 4 h beforehand had no significant analgesic activity even at doses of 1 and 10 mg/kg i.v. in the acetic acid writhing test in rats. In mice, the significant reduction in writhes counts was seen when dexamethasone (1 and 10 mg/kg i.v.) was given 15 min or 4 h before phenylquinone injection; i.e. the activity had not the lag time. On the other hand, dexamethasone showed a strong antipyretic activity against both the fevers caused by LPS and yeast in rats. In the yeast-febrile rats, the antipyretic activity had a lag time of about 1 h, and was dose-related at doses as low as 0.03 to 0.3 mg/kg i.v.; the steriod markedly reduced the increased PGE_2 content in the cerebrospinal fluid. The antipyretic activity after local injection into the cerebroventricle or the yeast pouch was stronger than that after systemic injection into the tail vein, although so large a difference in the activity between the dosage routes was not seen, suggesting that the site of the antipyretic action is in both the brain and periphery. The antipyretic activity of dexamethasone (10 mg/kg i.v.) was not seen in rabbits with fever caused by LPS. These results suggest that dexamethasone failed to produce the clear analgesic and antipyretic actions. The relation of the present result to the phospholipase A_2 inhibition by the steroid was discussed.

Introduction

It is considered that prostaglandins are chemical mediators or modulators for inflammation, pain and fever and that aspirin-like non-steroidal anti-inflammatory drugs (NSAIDs) produce their anti-inflammatory, analgesic and antipyretic action through the prevention of prostaglandin generation due to inhibition of cyclo-oxygenase activity [1–3]. The anti-inflammatory action of NSAIDs, however, is much weaker than that of anti-inflammatory steroids such as dexamethasone. The hypothesis is proposed for explaining the difference in anti-inflammatory potency between NSAIDs and the steroids; i.e. the steroid inhibits both the generation of prostaglandins and leukotrienes via the production of macrocortin or lipomodulin, specific protein which prevents precursor fatty acid release from intracellular lipid stores by inhibition of phospholipase A_2 [4–6]. Moreover, it has been reported that the steroids inhibit the prostaglandin synthesis, but do not prevent the cyclo-oxygenase [6, 7]. These observations suggest the possibility that the steroids may have the analgesic and antipyretic actions as potent as the anti-inflammatory action. However, there is a little investigation on the analgesic and antipyretic actions of the steroids. Thus, in order to explore the role of phospholipase A_2 inhibition in the mechanisms of anti-inflammatory action of the steroid, analgesic and antipyretic activities of dexamethasone were investigated in experimental animals.

Materials and methods

Analgesic assay

Male Wistar rats weighing 100 to 110 g were used in the acetic acid writhing test. Each rat was given intraperitoneally 1 ml of a 1% acetic acid aqueous solution and was settled into a cylindrical cage (24.5 cm in diameter) made of transparent acrylic resin. The number of writhes was counted for 20 min beginning from 60 min after acetic acid injection. Drugs were administered intravenously to only the rats, showing a writhing syndrome within 15 min after acetic acid injection, 15 min before the start of writhes counting; or to rats 240 min before the start of writhes counting [8, 9].

Female Std:ddY mice weighing 18 to 22 g were used in the phenylquinone writhing test. Each mouse was given intraperitoneally 0.1 ml/10 g body weight of a 0.03% phenylquinone solution in 5% aqueous ethanol, and the number of writhes was counted for 15 min beginning from

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5 min after phenylquinone injection. Drugs were administered intravenously 15 or 240 min before phenylquinone injection [9].

A tail flick response was induced by heat radiation on the blackened tail of a male mice (9–12 g) of ddN strain using an apparatus and procedure as described by NAKAMURA and SHIMIZU [10]. Mice showing a response time of 4 to 6 sec were used. After intravenous administration the response time was measured 8 times at 60 min intervals with an arbitrary cut-off time of 15 sec. Eight mice were used for each dose.

Antipyretic assay

Male albino rabbits weighing 2.5 to 4 kg were used. Each animal was placed in a cage which was kept at a constant room temperature of $24 \pm 1°C$ throughout the test. The rectal temperature was monitored by means of a thermistor probe (Takara Thermistor K-700, Takara Ind., Japan) and was recorded automatically at 5 min intervals for 7 h after intravenous injection of 1 μg/kg of LPS (lipopolysaccharide B., *E. Coli* 026 : B_6, Difco). Drugs were administered intravenously 4 h before or 1 h after LPS injection [11].

Male Wistar rats (350–500 g) were given subcutaneously 15 ml/kg of a 15% sterilized dry yeast aqueous suspension. Seventeen hours later, drugs were administered intravenously, subcutaneously or intracerebroventricularly only to rats showing an increase of 1°C or more in rectal temperature. Rectal temperature was measured 1 h before (pre-drug value) and hourly for 5 or 8 h after drug administration with a thermistor probe (Natsume, Japan) [9]. In the other group, drugs were injected directly into the yeast pouch; the yeast pouch was prepared by injecting the yeast suspension described above into the air pouch which was prepared by a subcutaneous injection of air (10 ml) 24 h before yeast injection. LPS-fever was caused by an intravenous injection of 20 μg/kg of LPS to rats.

Intracerebroventricular injection

Male Wistar rats (200–250 g) were used. A guide cannula was stereotactically implanted into the lateral ventricle of the brain under hexobarbital sodium anaesthesia, and at least 2 weeks later, the rats were used for the antipyretic assay. After intracerebroventricular (i.c.v.) administration of the test drug, the injected site was confirmed by injecting Evan's blue dye in the same manner.

PGE_2 measurement

The rat cerebrospinal fluid (10 μl) taken under ether anaesthesia was poured into the ice-cold test tube containing 1 or 0.2 ml of the assay buffer for RIA kit and the tube was frozen until use.

A PGE_2 radioimmunoassay kit (New England Nuclear, USA), based on the use of an ^{125}I-analogue of PGE_2 as tracer and rabbit anti-PGE_2 as specific antibody, was used. Due to the high concentration of this prostaglandin in the cerebrospinal fluid of the febrile rat, it was determined without any previous extraction procedure.

Statistical analysis

Student's *t*-test was used for statistical analyses.

Drugs

Drugs used were as follows: dexamethasone phosphate (Decadron® phosphate injection, Nippon Merck Banyu) and betamethasone phosphate. The test drug was dissolved in or diluted with aseptic saline for dosing. Doses are represented in terms of each salt.

Results

Effect of dexamethasone on nociceptive responses

It has been reported that anti-inflammatory activity of glucocorticoids becomes evident with a lag time of 1 to 2 h after dosage, since they exhibit the action after inducing the production of specific protein through the activation of gene expression [6, 12, 13]. Therefore, in one experiment, dexamethasone was administered 4 h before the start of writhes counting.

In the rat acetic acid writhing test, dexamethasone showed only weak inhibitory activity at doses of 1 and 10 mg/kg i.v.; the effect was significant when given 15 min before counting, but the maximum inhibitory rate was only 34.8% (Fig. 1A). In the mouse phenylquinone writhing test, dexamethasone caused a reduction in writhing counts at doses of 1 and 10 mg/kg i.v; the

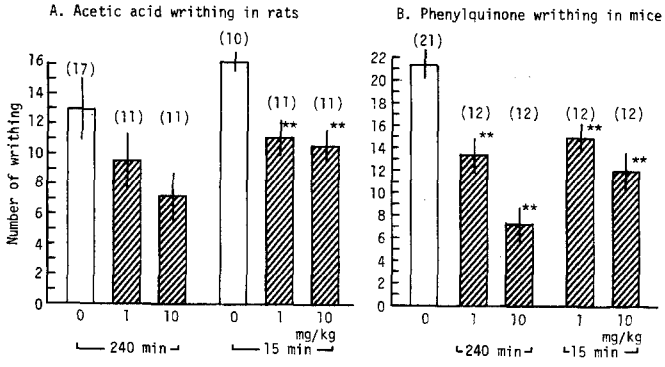

Figure 1
Effect of dexamethasone on writhing syndrome caused by acetic acid or phenylquinone in rats or mice. A: Dexamethasone was administered intravenously to rats 15 or 240 min before the start of writhes counting; number of writhes was counted for 20 min beginning from 60 min after acetic acid injection. B: Dexamethasone was administered intravenously to mice 15 or 240 min before phenylquinone injection; number of writhes was counted for 15 min beginning from 5 min after phenylquinone injection. Each column and vertical bar represent the mean and SEM. ( ) No. of animals used. $^{**}p < 0.01$ Significantly different from each control group.

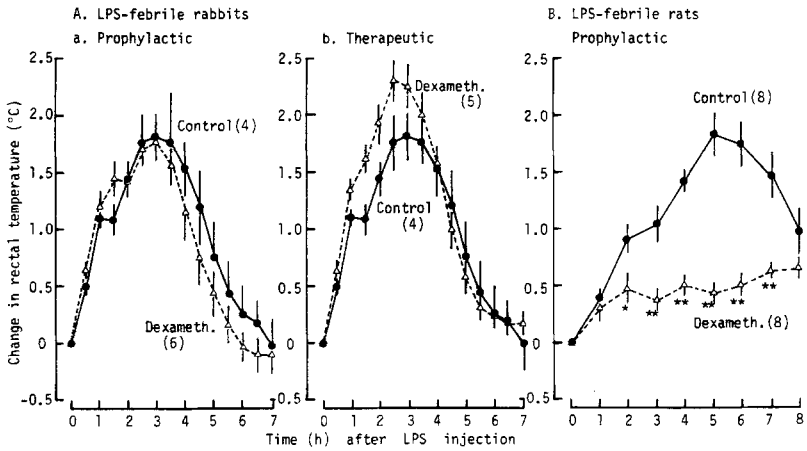

Figure 2
Effect of dexamethasone on LPS-induced fever in rats and rabbits. A: Dexamethasone (10 mg/kg) was administered intravenously to rabbits 4 h (a) before or 1 h (b) after LPS (1 μg/kg i.v.) injection. B: Dexamethasone (10 mg/kg) was administered intravenously to rats 4 h before LPS (20 μg/kg i.v.) injection. Each point and vertical bar represent the mean and SEM. ( ) No. of animals used. *$0.01 < p < 0.05$ and **$p < 0.01$ Significantly different from each control group.

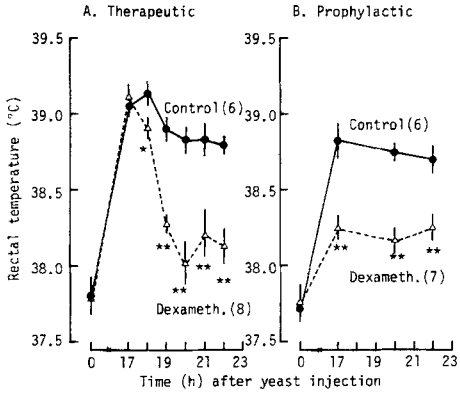

Figure 3
Effect of dexamethasone on yeast-induced fever in rats. Dexamethasone, 10 mg/kg or 5 mg/kg × 2, was administered intravenously to rats 17 h after (A) or 4 and 6 h before (B) subcutaneous injection of yeast suspension, respectively. Each point and vertical bar represent the mean and SEM. ( ) No. of rats used. *$0.01 < p < 0.05$ and **$p < 0.01$ Significantly different from each control group.

maximum inhibitory rate was 66.2% when given 10 mg/kg 4 h before phenylquinone injection, but was 38.0% at 1 mg/kg (Fig. 1B). In the mouse tail flick test, dexamethasone (1 and 10 mg/kg i.v.) did not show any effect on the tail flick response time; the mean time (sec) and SEM for 10 mg/kg of dexamethasone were 4.89 ± 0.20, 4.56 ± 0.26, 4.66 ± 0.26 and 4.61 ± 0.25 ($n = 8$) just before and 2, 4 and 8 h after dosing, respectively.

Effect of dexamethasone on fever caused by LPS

As shown in Fig. 2, dexamethasone (10 mg/kg i.v.) when given 4 h before or 1 h after LPS did not show any significant effect on fever caused by intravenous injection of LPS in rabbits. On the contrary, the same dose of dexamethasone produced a strong, longlasting inhibition of fever caused by LPS in rats (Fig. 2B).

Effect of dexamethasone on fever caused by yeast

Since dexamethasone showed a strong antipyretic activity to LPS-induced fever in rats, the effect of the steroid on fever caused by another pyrogen, yeast was tested in rats. As shown in Fig. 3, dexamethasone (10 mg/kg i.v.) also inhibited markedly the yeast fever by both the therapeutic and prophylactic treatments. In the therapeutic test (Fig. 3A), the effect at 1 h after dosage was significant but much weaker than that at 2 to 5 h after dosage, and the maximum effect was obtained 3 h after dosage.

In the therapeutic test, the PGE_2 level in the cerebrospinal fluid was measured by radioimmunoassay 5 h after dosage. Dexamethasone decreased markedly the increased PGE_2 level caused by yeast (Fig. 4).

The antipyretic activity of dexamethasone when given therapeutically was dose-related at dose range of 0.03 to 0.3 mg/kg i.v.; and the significant activity was found at a dose as low as 0.1 mg/kg. A similar dose–response relationship was obtained with betamethasone (Fig. 5).

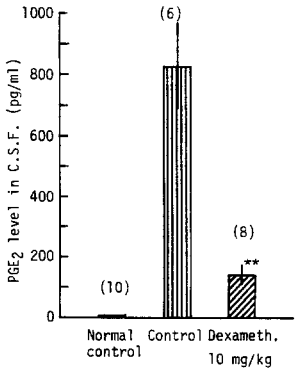

Figure 4
Effect of dexamethasone on PGE_2 level in the cerebrospinal fluid in the yeast-induced febrile rats. PGE_2 content was determined 5 h after intravenous injection of dexamethasone by radioimmunoassay in the febrile rats which were given subcutaneously yeast suspension 17 h before (see Fig. 3 explanations).

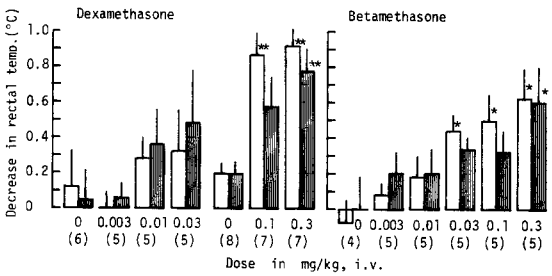

Figure 5
Comparison of antipyretic activities of dexamethasone and betamethasone in the yeast-induced febrile rats. Drugs were administered intravenously to the febrile rats 17 h after yeast injection; rectal temperature was measured hourly for 8 h after dosage. Open and shaded columns represent the decrease from the pre-drug value 5 and 8 h, respectively, after dosage. ( ) No. of rats used. *$0.01 < p < 0.05$ and **$p < 0.01$ Significantly different from each control group.

In order to explore the site of the antipyretic action of the steroid, antipyretic activities of betamethasone administered intravenously, intracerebroventricularly and subcutaneously (into the yeast pouch) were compared; betamethasone was used in this test since dexamethasone used was a commercial injection, and contained some solvents other than saline. Betamethasone showed a significant antipyretic activity at an intravenous dose of 0.03 mg/kg but not 0.01 mg/kg (Fig. 5). Therefore, 0.01 mg/kg of betamethasone was given to the rats 17 h after yeast injection. Rats were divided into two groups; each rat of the first and second groups received both the i.v. and s.c.

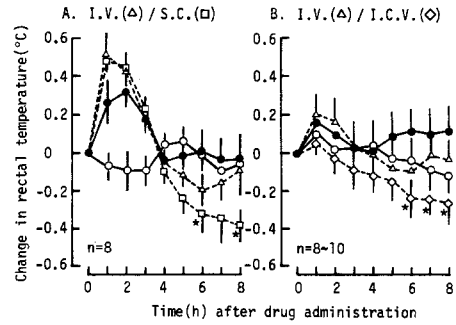

Figure 6
Comparison of antipyretic activities of betamethasone administered intravenously, subcutaneously and intracerebroventricularly in the yeast-induced febrile rats. Betamethasone (10 μg/kg) was administered intravenously (△), subcutaneously (into the yeast pouch; □) or intracerebroventricularly (◇), 17 h after yeast injection. The control rats (●) received saline both i.v. and s.c. (A), or both i.v. and i.c.v. (B). The betamethasone-treated groups were as follows: A. saline i.v. and saline s.c. (●), betamethasone i.v. and saline s.c. (△), betamethasone s.c. and saline i.v. (□); B. saline i.v. and saline i.c.v. (●), betamethasone i.v. and saline i.c.v. (△), betamethasone i.c.v. and saline i.v. (◇). The normal control group (○) received no treatment. Each point and vertical bar represent the mean and SEM. The ordinate represents the change from the value at 17 h after yeast injection. *$0.01 < p < 0.05$ Significantly different from each saline control group.

injections, and both the i.v. and i.c.v. injections, respectively, of betamethasone and saline, saline and betamethasone, or saline and saline. Betamethasone did not produce any significant antipyretic activity after i.v. administration in either of the groups (Fig. 6). However, the effects after s.c. and i.c.v. administration were significant in comparison with each saline control group, although were not significantly different from each i.v. administered group; both the activities were approximately equal. An increase in body temperature during 3 h after dosage in the first group (Fig. 6A) was probably caused by manipulation of the yeast pouch for dosing. These results suggest that betamethasone shows stronger antipyretic activity after s.c. or i.c.v. administration than that after i.v. administration.

Discussion

Glucocorticoids act as a consequence of their binding to cytoplasmic receptors, followed by the translocation of the ligand–receptor complex into the nucleus, which, in turn, affects

the transcription of various RNA species. Thus, there is a lag time between administration of the drug and onset of the action. In the histamine-induced inflammation in rat skin, when given 1 h before histamine dexamethasone has no significant activity, and the maximum inhibition is observed 4 h after dosage; the lag time is less than 4 h in the carrageenin paw edema test in rats [12]. In mice, dexamethasone when given 1 h before phlogistics has no significant effect on hind paw edema caused by serotonin or bradykinin, and the maximum inhibition is observed 3 h or more after dosage [13, 14]. In these tests, anti-inflammatory ED_{50}-values of dexamethasone are 0.01 to 0.1 mg/kg. In the *in vitro* test, dexamethasone inhibits almost completely at 10^{-6} *M* and partially at 10^{-8} *M* PGE_2 production and its IC_{50} is 0.3×10^{-8} *M* [7, 15]. These observations demonstrate that dexamethasone produces the anti-inflammatory action with a lag time of at least 1 h at doses of up to 0.1 mg/kg in mice and rats. This appears to be a typical mode of the anti-inflammatory action of dexamethasone.

Dexamethasone when given 4 h beforehand had no significant activity even at doses of 1 and 10 mg/kg in the acetic acid writhing test which readily detects NSAIDs in rats. In mice, the significant reduction in writhes counts was seen when dexamethasone was given 4 h before phenylquinone injection. The activity, however, was not significantly different from that when given 15 min beforehand. Dexamethasone, like NSAIDs, had no analgesic activity in the tail flick test which readily detects narcotic analgesics. Subsequently, dexamethasone failed to produce the analgesic action in a manner similar to the typical anti-inflammatory action.

Dexamethasone showed a strong inhibitory activity against both the fevers caused by LPS and yeast in rats. Its antipyretic activity had a lag time of about 1 h; and was dose-related at doses as low as 0.03 to 0.3 mg/kg. Furthermore, dexamethasone markedly reduced the increased PGE_2 content in the cerebrospinal fluid. This mode of the antipyretic action resembled closely to that of the anti-inflammatory action of dexamethasone. On the other hand, the antipyretic activity after local injection into the cerebroventricle or yeast pouch was stronger than that after systemic injection into the tail vein, although so large difference in the activity between i.c.v. or s.c. and i.v. administrations was not seen, suggesting that the site of the antipyretic action is in both the brain and periphery. It has been reported that cortisol produces a decrease in endogenous pyrogen production from leucocytes *in vitro* [16]. Thus, a part of the antipyretic action of the steroid might be due to the inhibition of endogenous pyrogen production and/or of leucocytes emigration into the yeast pouch, although NSAIDs produce its antipyretic action through the central mechanism [3].

On the contrary, the antipyretic activity of dexamethasone was not seen in rabbits with fever caused by LPS. It has been reported that cortisol when given repeatedly to rabbits inhibits the febrile effect of bacterial pyrogen [17, 18]. WILLIES and WOOLF [19] reported that intravenous infusions of hydrocortisone or methylprednisolone, when administered simultaneously with bacterial or endogenous pyrogens, failed to produce an antipyresis, although pretreatment with methylprednisolone for 3 days diminished both the febrile responses in rabbits. However, the direct injection of cortisol or methylprednisolone into the preoptic region or hypothalamus of rabbits partially but significantly prevents the fever caused by bacterial or endogenous pyrogen [19, 20]. The present result with a potent glucocorticoids, dexamethasone confirmed that the febrile effect of bacterial pyrogen is not influenced by the systemically single treatment with the steroids in rabbits. Glucocorticoid receptors exist in the brain of the rabbit as well as the rat, mouse and human [21–24]. On the other hand, it has been reported that NSAIDs have the organ selectivity in the cyclo-oxygenase inhibition [25, 26]. Thus, for explaining the low sensitivity of rabbits to the steroid, it is presumed that there may be relatively a little glucocorticoid receptor in the rabbit brain, or the phospholipase A_2 in the rabbit brain may be less sensitive to the steroid. Further work is required to elucidate the mechanism involved in the steroid antipyresis in rabbits. At all events a high dosage of dexamethasone failed to produce a significant antipyretic action in the LPS-febrile rabbit test which readily detects NSAIDs.

In the present experiments, the typical dexamethasone-like action was seen in the antipyretic action in rats, but was not seen in the antipyretic action in rabbits and in the analgesic action in mice and rats. Consequently, these results suggest that the phospholipase A_2 inhibition by the steroid may be one out of the many mechanisms of the action of the steroid.

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