Biochemical Pharmacology, Vol. 26, pp. 1613–1617. Pergamon Press, 1977. Printed in Great Britain.

FEBRILE RESPONSE AND VARIATIONS IN THE BRAIN LEVELS OF BIOGENIC AMINES AND THEIR METABOLITES IN RABBITS MADE TOLERANT TO *E. COLI* LIPOPOLYSACCHARIDE

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(*Received* 19 *October* 1976; *accepted* 21 *February* 1977)

Abstract—Rabbits made tolerant to bacterial pyrogens by repeated daily i.v. injections of 1.5 μg/kg *E. coli* LPS, recovered their full reactivity to fever when reinjected with LPS on day 12 after a 1-week resting period. Afebrile animals showing induced tolerance (sacrificed on day 5 after 4 daily LPS injections) had increased levels of NA and decreased levels of DOPAC and HVA in hypothalamus and brain stem. These changes are viewed as a long-term adaptive mechanism in response to the stress elicited by repeated LPS injections. In both febrile and afebrile animals lower NA and higher HVA levels were found in the hypothalamus 3 hr after a single LPS injection, or after the last of five daily LPS injections, or after a fifth LPS injection given after a resting period of 1 week following four daily LPS injections. As these changes took place independently of fever they are held to be the manifestation of a short-term adaptive response of the organism to a febrile trauma.

In 1892, Klemperer [1] reported that repeated daily injections of bacterial pyrogen induced a state of tolerance in dogs and rabbits. Later, various authors observed this type of decreased febrile reponse following successive administrations of bacterial pyrogens—either non-purified, such as antimicrobial vaccines, or purified, such as bacterial lipopolysaccharides. The experiments have been reviewed by Bennett and Cluff [2] and Atkins [3].

This study presents some observations on the febrile response and the induced tolerance to *E. coli* lipopolysaccharide (LPS) following repeated daily, i.v. injections in rabbits and the effects of this type of tachyphylaxis on the levels of biogenic amines and their metabolites found in the tissues of various brain structures. The purpose of the present experiments was to find out (a) if the condition of induced tolerance modifies the metabolism of the brain biogenic amines and (b) if induced tolerance provides a model in which the febrile response has become dissociated from the biochemical changes observed previously [4] during the febrile response.

MATERIALS AND METHODS

Male mongrel rabbits with a mean body weight of 2.5 kg were housed under controlled conditions of temperature (20° ± 1) for one week prior to use and during the 5 or 12 days period of experimental procedure.

Rectal temperature was measured with a 'Ellab' thermocouple (Electrolaboratoriet, Copenhagen) every 15 min for 1 hr before and up to 7 hr after each injection.

All results were obtained in groups of ten animals. The treatment of the groups varied according to the number of injections of either LPS or 0.9% saline solution (control groups) and according to the time of sacrifice after the last injection. Some groups received daily injections of either LPS or of 0.9% saline for 4 consecutive days, being sacrificed 1 day later, or for 5 consecutive days being sacrificed 1.5 hr or 3 hr after the last injection. Other groups received daily injections of either LPS or 0.9% saline for 4 consecutive days and were then given a rest period of 7 days when they were either sacrificed without further treatment or given a further LPS injection and sacrificed 1.5 or 3 hr later.

The preparation, storage, and dilution of the *E. Coli* LPS solution has been described elsewhere [4]. The dose given was 1.5 μg/kg i.v. per day. Pyrogen free 0.9% saline solution (1 ml/kg) administered i.v. served as a control. All injections were given at approximately 10:00.

Biochemical analysis. The procedures for sampling the tissues, extraction, and assay for the amines and their metabolites have previously been described in detail [4]. In each case, the concentrations were calculated by internal standard.

The concentrations of the following compounds were determined in the hypothalamus (H) and the brain stem (BS). Serotonin (5-HT), dopamine (DA), noradrenaline (NA) and their respective metabolites 5-hydroxyindolacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MOPEG-SO_4), the major metabolite of NA in the brain. In addition, measurements were made of the levels of DA and its metabolites in the caudate nucleus (CN) and of 5-HIAA, HVA and MOPEG-SO_4 in the cerebrospinal fluid (CSF).

Statistical significance was assessed by using Student's *t*-test. The results were expressed as the mean ± S.E.M.

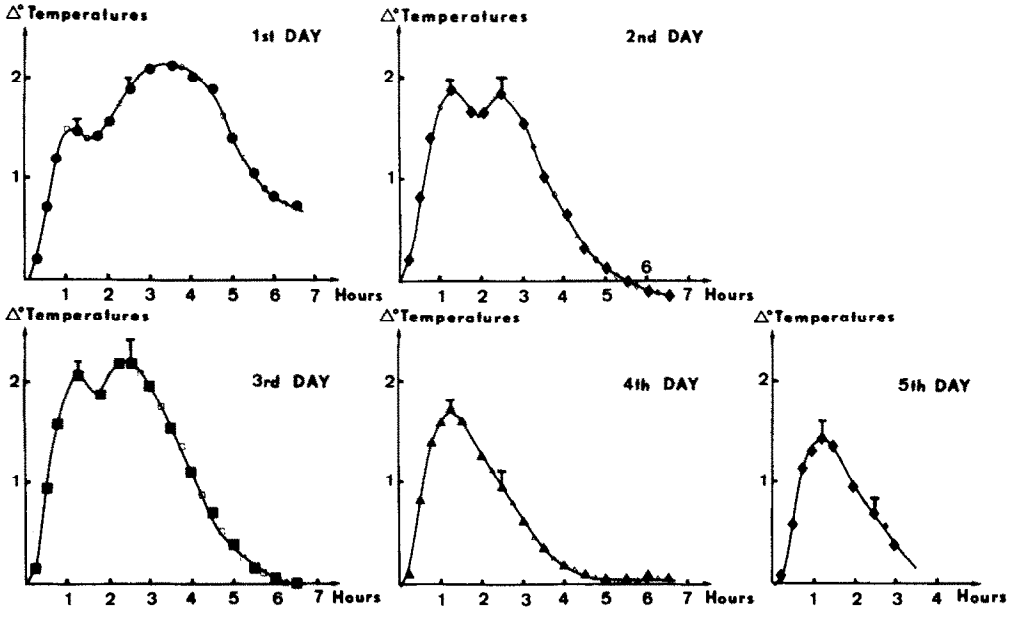

Fig. 1. Daily changes in the rectal temperature of rabbits receiving repeated daily i.v. injections of *E. coli* LPS (1.5 μg/kg).

RESULTS

Febrile response to LPS

Following the administration of a single LPS injection, the febrile response in the rabbit is characteristically biphasic (Fig. 1, 1st day). Two peaks occur at approximately 1.5 hr and 3–3.5 hrs post-administration. Therefore, the time points of 1.5 hr and 3 hr were chosen as yielding the most fruitful data for the present study on the effects of repeated injections of LPS.

Daily injections for 4 or 5 days. A progressive increase occurred in the magnitude of the first peak for the first 3 days. After the 3rd injection, the first peak was significantly higher ($P \leqslant 0.05$; Fig. 1) than after the 1st injection. After the fifth injection, the first peak was not significantly different from the one obtained after the first injection.

A progressive reduction occurred of the second peak during the first three injections and the duration of fever decreased. After the fourth injection, the second peak had disappeared and the febrile response had become monophasic. Three hr after the fifth LPS injection, temperature did not differ from normal values (Fig. 1).

Daily LPS injection for 4 days, followed by a 1-week resting period; then a 5th LPS injection on day 12. Following re-injection on day 12, the febrile response returned to its original biphasic form. However, the curves were not identical to those found on day 1: for the 22 animals receiving this treatment, three showed a first peak which was lower than that of day 1; and 19 animals showed a first peak which was the same or mostly higher than that of day 1. Table 1 shows the average rise in temperature for day 12 as compared to days 1 and 2. It can be seen that the value of the first peak on day 12 was similar to the one obtained for the first peak on day 2, and that the value of the second peak was comparable to the value of the second peak obtained on day 1.

Furthermore, a comparison of the duration of fever on day 12 with that on days 1 and 2 shows that there was a decrease in relation to day 1 and an increase in relation to day 2. These relationships are better expressed by means of a planimetric fever index (F.I.) at 6 hr post administration. If a value of 100 is given to F.I. on day 1, then F.I. is 55 on day 2 and 85 on day 12.

Effects on levels of biogenic amines and their metabolites

Daily LPS or 0.9% injections for 4 days; animals sacrificed on day 5. As shown in Fig. 2, the LPS-treated rabbits showed no change, compared to the control group, in the levels of 5-HT or DA in the hypothalamus (1,432 ± 79 ng/g and 435 ± 19 ng/g respectively), in the brain stem (1,715 ± 89 ng/g and

Table 1. Comparison of febrile responses (mean ± S.E.M.) obtained with rabbits treated with LPS. The values are measured at specific time intervals (corresponding to the first and second peaks) following the 1st injection and repeated injections

LPS treatment schedules‡	Febrile state: ΔT(°C) 1.5 hr	3 hr
Day 1: after 1st injection	1.30 ± 0.09 (22)	1.95 ± 0.14 (22)
Day 2: after 2nd injection	1.67 ± 0.08† (22)	1.50 ± 0.17* (22)
Day 12: reinjection after 4 consecutive injections + resting period	1.78 ± 0.08† (22)	2.05 ± 0.18 (14)

‡ The experimental procedures are described in the text.
( ) Mean number of animals.
* different ($p \leqslant 0.05$) when compared to day 1.
† different ($p \leqslant 0.01$) when compared to day 1.

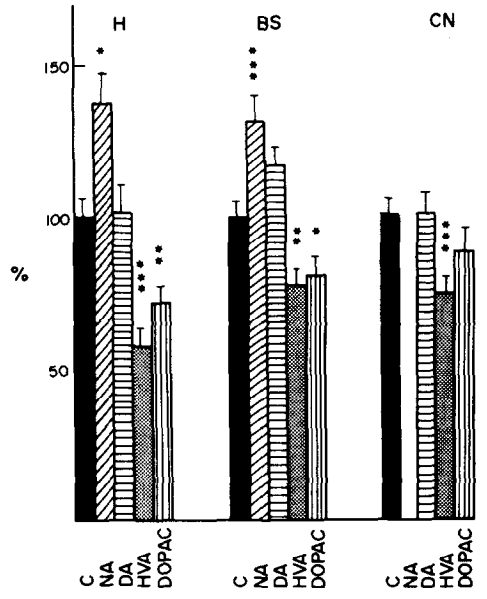

Fig. 2. Comparison of the levels of NA, DA, HVA and DOPAC in the hypothalamus, brain stem and caudate nucleus of control rabbits and rabbits receiving 4 daily i.v. injections (1.5 μg/kg) of LPS; the treated animals were sacrificed on day 5. The significance levels are as follows: *P < 0.05, **P < 0.01, ***P < 0.001.

206 ± 6 ng/g respectively), or in the concentration of DA in the caudate nucleus (13,08 ± 0,58 μg/g). However, the NA levels were significantly higher both in the hypothalamus and the brain stem (control values 1,724 ± 153 and 458 ± 23 ng/g respectively).

The concentrations of 5-HIAA and MOPEG-SO_4 were unchanged in the hypothalamus (1,520 ± 78 ng/g and 296 ± 20 ng/g respectively) and brain stem (1,945 ± 57 ng/g and 217 ± 15 ng/g respectively), however the levels of DOPAC and HVA decreased significantly both in the hypothalamus (control values: 174 ± 12 ng/g and 1,097 ± 70 ng/g respectively) and in the brain stem (control values: 95 ± 5 ng/g and 506 ± 20 ng/g respectively). In the caudate nucleus the concentration of HVA was also significantly decreased, but the DOPAC level was unchanged (control values: 5,280 ± 290 ng/g and 1,579 ± 94 ng/g respectively).

In the CSF no significant differences were found after the LPS injections in the levels of 5-HIAA (68 ± 3 ng/ml) HVA (48 ± 5 ng/ml) and MOPEG-SO_4 (44 ± 6 ng/ml).

Daily LPS injections for 5 days; animals sacrificed 1.5 hr or 3 hr after the last injection. The results are summarized in Fig. 3. They have been compared with the results shown in Fig. 2. No changes were found in the levels of 5-HT and DA in hypothalamus and brain stem 1.5 hr and 3 hr after the last injection but in the group of animals killed 3 hr after the last injection the NA level in the hypothalamus was significantly decreased. As regards metabolites only the levels of HVA became significantly increased in the hypothalamus and brain stem 1.5 hr and 3 hr after the last injection. In the caudate nucleus there were no changes in the levels of DA and DOPAC, nor in the level of HVA.

Daily LPS or 0.9% saline injections for 4 days followed by a one week resting period; animals either sacrificed or given a 5th LPS injection and then sacrificed. Figure 4 shows the results after a resting period of 1 week following four daily injections of LPS.. Only a slight decrease was still present in the HVA level of the brain stem, but there was also a small decrease in the level of DOPAC when compared to the controls. The results obtained in the group of animals which received a fifth LPS injection after the 1 week resting period are summarized in Fig. 5. They have to be compared with the results shown in Fig. 4. The main changes observed were a significant decrease in the NA concentration of the brain stem 1.5 hr and 3 hr, and of the hypothalamus 3 hr after the injection; 1.5 hr after the injection the reduction in the NA concentration in the hypothalamus was statistically not significant. There were statistically significant increases in the HVA levels at both time intervals, but only in the brain stem.

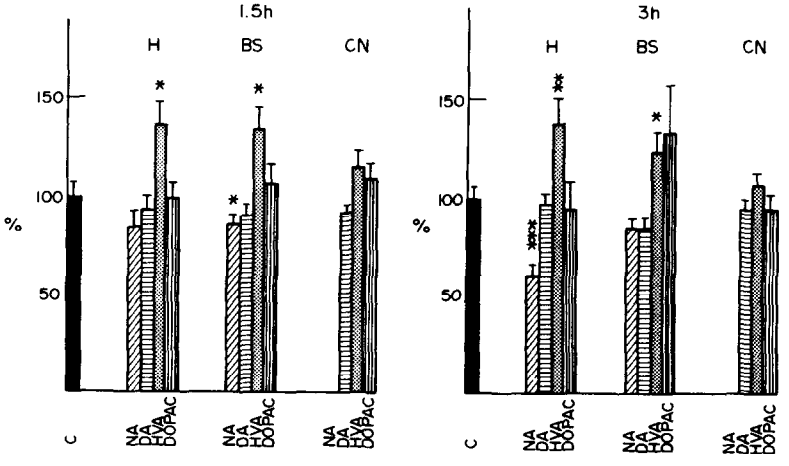

Fig. 3. Comparison of the levels of NA, DA, HVA and DOPAC in rabbits receiving daily i.v. injections of LPS as follows: (1) for 4 days, sacrificed on day 5 (C); (2) for 5 days, sacrificed 1.5 and 3 hr post-administration (see Fig. 2 for significance levels).

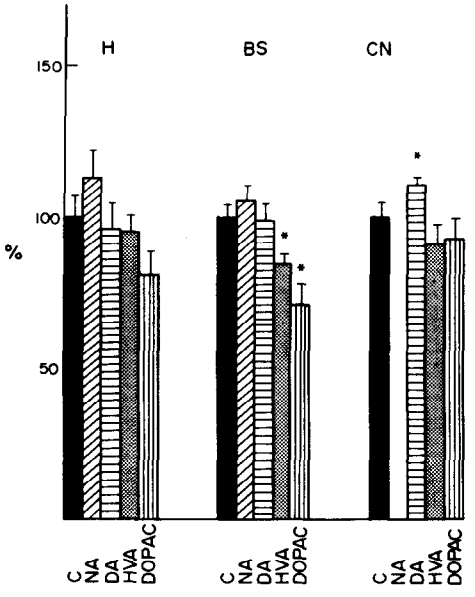

Fig. 4. Same as Fig. 2, but the animals were sacrificed on day 12, i.e. after a resting period of 7 days.

DISCUSSION

The febrile response in the rabbit to daily i.v. injections of LPS was found to become reduced both in magnitude and duration. Since a similar result has been obtained in the goat with LPS [5] and in the cat with lipid A [6] it would appear that tolerance induced by bacterial pyrogens is not species-linked. The most striking observation during the LPS-induced tolerance was the disappearance of a second fever peak which according to Greisman and Woodward [7] results from endogenous pyrogens derived from the liver, in response to the fourth daily injection. An immunological mechanism may be responsible for it since Root and Wolff [8] showed a similar tachyphylaxis in rabbits following daily injections not of an LPS but of an immune serum containing soluble antigen-antibody complexes.

After a 1-week resting period following a series of daily i.v. injections of LPS the rabbits were no longer tolerant and reverted to the original biphasic fever pattern. But there were differences between the response and that obtained with the first injection of LPS which are difficult to relate to the state of tolerance. For instance, no convincing explanation can be offered for the finding, not previously described, that the first peak of the fever response to LPS given after the 1 week resting period was usually higher than that of the response to the first LPS injection.

It is not possible at this stage to say whether the changes during the induced tolerance—the increase in the concentration of NA in the brain without a change in the level of its metabolite MOPEG-SO_4 and the decrease in the acid metabolites of DA without a change in the level of DA—are the cause or the result of the tachyphylaxis or whether the two phenomena are independent. However, the finding that the biochemical changes occur also when the LPS injection is given during the induced tolerance although temperature does not rise, shows that the changes in amine metabolism occur independent of the febrile response. Consequently the pyrogen-resistant rabbit provides a model for dissociating the febrile response from the changes produced by LPS in the levels of biogenic amines in the brain.

There may well be a connection between the increase in the level of NA in the brain during induced tolerance and the increase in the NA concentration observed during prolonged stress [9–11]. Furthermore, Thoenen [12] and Kvetnansky [13] found in *in vitro* experiments that the tyrosine hydroxylase activity of the suprarenal medulla and of adrenergic nerve endings is increased in a variety of experimental conditions leading to a state of prolonged general

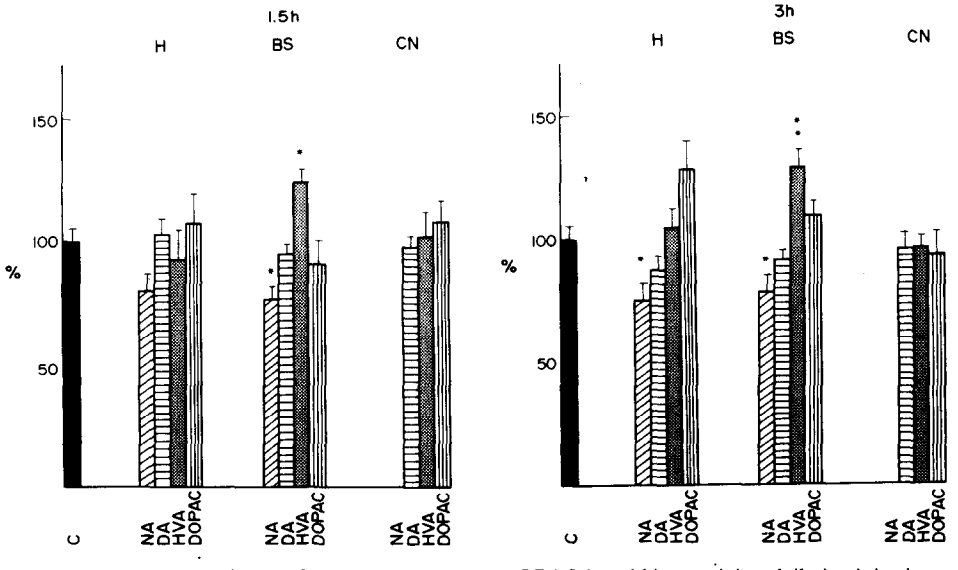

Fig. 5. Comparison of the levels of NA, DA, HVA and DOPAC in rabbits receiving daily i.v. injections of LPS as follows: (1) for 4 days and sacrificed on day 12 after a resting period of 7 days (C); (2) as above but sacrificed on day 12 at 1.5 and 3 hr after a 5th injection of LPS (1.5 μg/kg) (see Fig. 2 for significance levels).

stress. According to Thoenen the higher concentration of this enzyme at the active sites is probably the result of increased synthesis of its enzyme-protein. For instance, Spurr [14] reported that in dogs subjected for 5 days to repeated hyperthermias the level of serum glutamic-oxalo acetic transaminases, serum glutamic-pyruvic transaminase and isocitric dehydrogenase was increased.

It is difficult to explain the differences found between the response displayed by the metabolites of DA (a decrease in the level of DOPAC and HVA) and of NA (no change in the level of MOPEG-SO_4). It may be that the DA catabolism is preferentially directed to NA synthesis resulting in a fall of the DA metabolites, as if a partial inhibition of the responsible enzymes had taken place. The NA synthesized might then be taken up by the 'storage compartment' as Glowinski [15] called it. This author considers this NA pool an older form of the amine which is not readily released in contrast to the NA of the 'functional compartment' which takes up the newly synthesized NA and uses it preferentially during neuronal activity [16]. In this way no changes need to occur in the level of MOPEG-SO_4.

Viewed in connection with the biochemical changes produced in animals subjected to prolonged stress our results might be considered to be a manifestation of a long-term adaptive mechanism in response to the induction of tolerance to LPS. This mechanism would develop independently of the short-term response described earlier [4] which follows the administration of a single LPS injection.

There is also the possibility that the increase of NA in the hypothalamus and brain stem during induced LPS tolerance result from an interaction with prostaglandins, which would most likely be the E prostaglandins released during pyrogen-induced fever since according to the theory of Hedqvist [17] the E prostaglandins have a modulatory role in the noradrenergic nervous system.

Acknowledgement—The authors are indebted to Mrs Louise Monnier for technical assistance.

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