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Effects of Nicotine on Plasma Corticosterone and Brain Amines in Stressed and Unstressed Rats

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BALFOUR, D. J. K., A. K. KHULLAR AND A. LONGDEN. *Effects of nicotine on plasma corticosterone and brain amines in stressed and unstressed rats.* PHARMAC. BIOCHEM. BEHAV. 3(2) 179–184, 1975. – The administration of nicotine (0.4 mg/kg) to unstressed rats caused a rise in plasma corticosterone which persisted for 60 minutes and a fall in hippocampal 5-hydroxytryptamine (5-HT) at 45 minutes followed by a rise at 60 minutes. In rats which were stressed by being placed on an elevated platform, nicotine caused a reduction in hippocampal 5-HT at 45 and 75 minutes but did not affect the plasma corticosterone concentration. Rats studied 16 hours after the last injection of a course of treatment with metyrapone had much reduced levels of plasma corticosterone and hippocampal 5-HT. Under the present conditions metyrapone also much diminished the effects of nicotine on plasma corticosterone levels in unstressed rats but had little effect on the response to stress.

Nicotine Stress Hypothalamus Hippocampus Noradrenaline 5-Hydroxytryptamine Corticosterone Rat

RECENTLY Hall and Morrison [5] have reported that dependence on nicotine can develop in rats exposed to a stressful situation. These authors suggested that the mechanism through which dependence on nicotine may develop could involve the release of noradrenaline (NA) from the hypothalamus [6] resulting in a reduction in corticosteroid secretion from the adrenal cortex, This hypothesis was based on previous studies which have indicated that NA may serve an inhibitory function in the control of ACTH secretion from the pituitary gland [16]. Further evidence suggests that 5-hydroxytryptamine (5-HT) may also be involved in the regulation of ACTH release [13]. Nicotine has recently been shown to affect the uptake and release of NA and 5-HT by hypothalamus and hippocampus [2]. These observations are of interest since both these brain structures have been implicated in the control of ACTH secretion [11]. In the present study, an attempt has been made to relate changes in the levels of these biogenic amines in hypothalamus and hippocampus to the effects which nicotine exerts on plasma corticosterone concentrations in stressful and non-stressful situations.

METHOD

Animals

Male Sprague-Dawley rats (Olac) weighing approximately 200 g and housed in groups of 4 were used for these experiments.

Procedure

Injections of nicotine consisted of nicotine hydrogen tartrate (British Drug Houses) dissolved in 0.9% saline to give an injection volume of 0.1 ml/100 g body weight. The dose (0.4 mg/kg) is expressed as base. Metyrapone (CIBA) was dissolved in sterile saline (25 mg/ml) and rats received 0.2 ml/100 g. For the biochemical estimations, corticosterone, NA hydrochloride and 5-HT creatinine sulphate were supplied by Sigma; fluorescence reagent FDPC (for corticosterone assay) by British Drug Houses.

Nicotine. In order to reduce any stressful effects of a novel injection on the day of experiments, rats received daily subcutaneous injections of serile saline (0.1 ml/100 g) for 5 days prior to the experiment. On the day of experiment, the rats received a subcutaneous injection of nicotine or saline and were then returned to their home cages. At various times (15–90 min) after the injection, the rats were killed by decapitation and the brains rapidly removed. A blood sample (0.5 ml) was taken from the heart and placed in a small heparinised tube. The hypothalamic and hippocampal regions were dissected from the rest of the brain using a procedure similar to that of Glowinski and Iversen [3]. The tissues were weighed and homogenised in

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5 ml of cold acid butanol reagent [1] and stored overnight at −40°C before being processed.

All experiments were performed in the morning (09.30–12.00 hrs) when diurnal variation in plasma corticosterone is minimal [7,13]. In all experiments saline controls were run simultaneously.

Stress. Rats were psychologically stressed by placing them singly on an elevated platform consisting of a square piece of perspex (20.5 x 20.5 cm), covered with a metal grid and fixed to the top of a 160 cm pole. The platforms were sufficiently large to accommodate a rat without causing discomfort. Control rats were left in their home cages. After remaining on the platform for various times (15–120 min) the animals were killed by decapitation and the brains and plasma removed and processed as described in the previous section. In experiments where rats received injections of saline or nicotine before being placed on the elevated platforms, they received 5 daily injections of saline prior to the experiment, as described previously, and then, on the final day, an injection of saline or nicotine 5 min before being placed on the platform.

To examine the effects of nicotine on the recovery of plasma corticosterone levels following removal from the stressful situation, rats were given a subcutaneous injection of nicotine or saline and were then placed on the platforms for 30 min. They were then returned to their home cages for a further 30 min before being sacrificed. A blood sample was then obtained. Nonstressed saline-treated controls remained in their home cages for the entire 60 minutes.

Metyrapone. Metyrapone (50 mg/kg), a specific inhibitor of 11-hydroxycorticosteroid synthesis [4, 8, 10], was injected by the intraperitoneal route at 16.30 hours for 3 consecutive days. The rats were killed on the following morning between 09.00 and 10.30 hours. Control rats received intraperitoneal injections of saline. Rats which received an additional subcutaneous injection of nicotine or saline before being killed were also given 5 daily subcutaneous injections of saline at 09.30 hours prior to the experiments as described previously.

Biochemical estimations. Plasma corticosterone was estimated using a method similar to that of Mattingly [12]. Corticosterone in a 0.1 ml sample of plasma was extracted into 0.75 ml of dichloromethane, and 0.5 ml of the dichloromethane solution was reacted with 0.5 ml of the acid-ethanol fluorescence reagent. The fluorescence was measured 90 minutes later in an Aminco Bowman Spectrofluorometer (excitation 470 nm and fluorescence 535 nm). The results were compared with the readings for corticosterone standards which had been taken through the extraction procedure. No correction was made for residual fluorescence. NA and 5-HT were estimated using the method of Ansell and Beeson [1] but with the following modifications: (a) After adsorption of the NA onto alumina, the alumina was washed with 0.05 M acetate buffer (pH 7.0), in place of distilled water, prior to the elution of the NA. This change was found to give higher final recoveries of NA. (b) In the estimation of 5-HT the mixed tissue blank, recommended by Ansell and Beeson [1] and which, in our hands, gave the same fluorescence as the unextracted blank, was replaced by a blank prepared by going through the extraction in the absence of any tissue and reacting the resulting extract with ninhydrin in the same way as the other tissue extracts. This blank, which gave somewhat higher values than the others, seemed to us to be more valid.

Under the present conditions the recovery of 5-HT was 70 percent and of NA 85 percent.

Statistics. Results were compared by two factor analysis of variance [15].

RESULTS

Over the time period used for these experiments (09.00 to 12.00 hours) the levels of plasma corticosterone and brain amines showed no diurnal variation.

The administration of nicotine (0.4 mg/kg) caused an elevation of plasma corticosterone which persisted for 60 minutes after the injection (Fig. 1). Thereafter the levels fell sharply and the difference was no longer significant at 75 min. No changes in the brain amine levels were observed until 45 min after the nicotine injection (Fig. 1) when there was a significant ($p<0.05$) reduction in the hippocampal 5-HT concentration. At 60 minutes hippocampal 5-HT was significantly ($p<0.01$) elevated. Hypothalamic 5-HT and NA in both hypothalamus and hippocampus were found to be unaffected by nicotine and in most of the subsequent experiments these parameters were not measured.

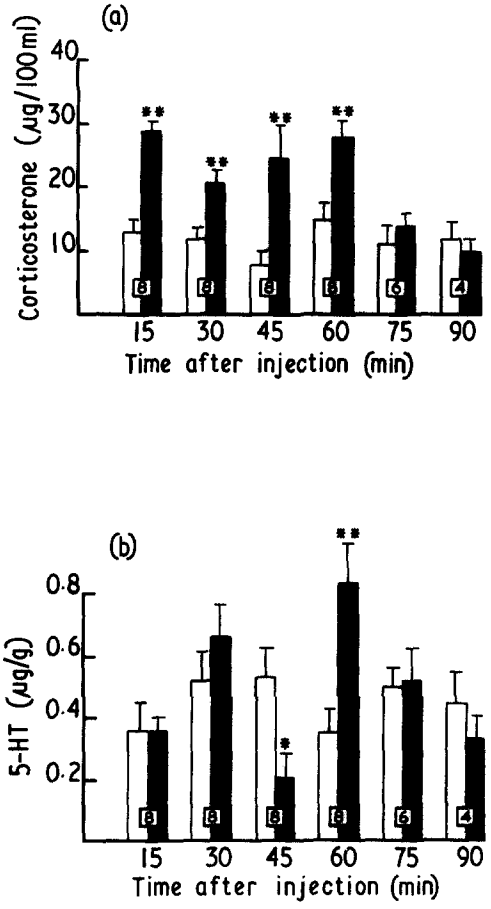

FIG. 1. Effects of nicotine (0.4 mg/kg) on plasma corticosterone (Fig. 1a) and hippocampal 5-HT (Fig. 1b) in unstressed rats. The results represent the means ± SEM of the number of observations given in the brackets. □ control, ■ nicotine-treated. *$p<0.05$, **$p<0.01$.

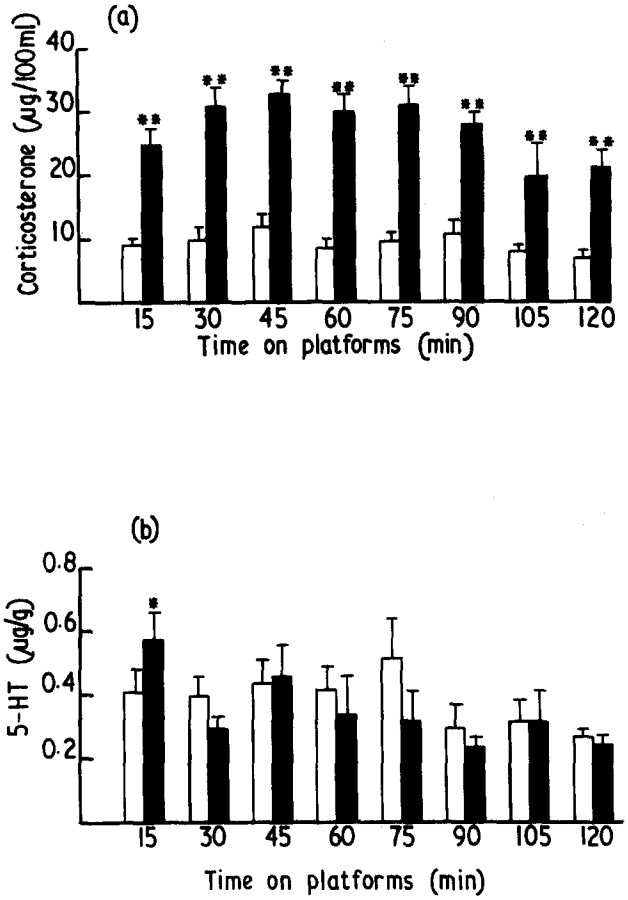

FIG. 2. Effects of stress on plasma corticosterone (Fig. 2a) and hippocampal 5-HT (Fig. 2b). The results represent the means ± SEM of 6 observations. □ control, ■ stress, *$p<0.05$, **$p<0.01$.

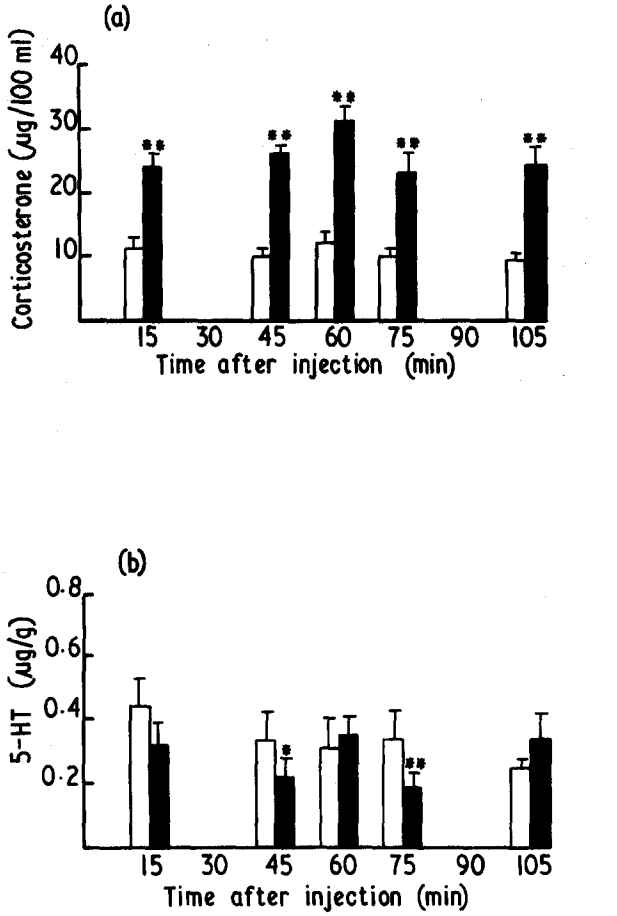

FIG. 3. Effects of nicotine (0.4 mg/kg) on plasma corticosterone (Fig. 3a) and hippocampal 5-HT (Fig. 3b) in stressed rats. The results represent the means ± SEM of 6 observations. □ control, ■ nicotine-treated, *$p<0.05$, **$p<0.01$.

The plasma corticosterone levels in rats placed on elevated platforms were increased throughout the period for which they remained on the platforms (Fig. 2). Thirty to 45 min elapsed before plasma corticosterone levels reached a maximum and 30 min later the levels showed a slight decline. Fifteen min after being placed on the platform, the hippocampal 5-HT concentration was significantly increased ($p<0.05$). Between 60 and 90 min the concentration of hippocampal 5-HT appeared to be reduced, but this response was not significant at any of the times studied.

Plasma corticosterone and hippocampal 5-HT levels in rats which had received nicotine before being placed on the platforms were measured and compared with saline-treated animals which remained in their home cages (Fig. 3). Plasma corticosterone levels were elevated during the whole course of the experiment (105 min). Hippocampal 5-HT was reduced at 15, 45 and 75 min, an effect which was significant at 45 and 75 min. In other experiments a direct comparison between the effects of saline and nicotine on the plasma corticosterone levels found in stressed rats was made. In these experiments two rats were injected simultaneously, one with nicotine the other with saline, and were then placed on separate platforms for 15 or 45 min before being killed and the plasma corticosterone estimated. The results (Table 1) show that nicotine was without effect on the raised corticosterone levels found in the plasma of stressed rats, when compared with their saline-treated partners at either of the times examined.

When rats were allowed to recover for 30 min in their home cages after spending 30 min on the platforms, plasma corticosterone levels of the saline-treated rats had almost returned to control levels (Table 2). However, if rats had received nicotine before being placed on the platforms, the plasma corticosterone concentrations after the 30 min recovery period were significantly higher than the values for the saline-treated group ($p<0.01$) as well as the unstressed control rats ($p<0.005$), and were not much less than the plasma corticosterone concentration found, in a previous experiment, in nicotine-treated unstressed rats (28 ± 4 μg/100 ml).

Injections of metyrapone caused a significant reduction in the plasma corticosterone concentration of 50 to 70 percent ($p<0.01$) and in the hippocampal 5-HT concentration of about 60 percent ($p<0.01$), but had no effect on the concentration of 5-HT in the hypothalamus. When

TABLE 1

EFFECTS OF NICOTINE AND STRESS ON PLASMA CORTICOSTERONE

Treatment	Plasma Corticosterone (μg/100 ml) 15 min	45 min
Saline Control	13.4 ± 2.1	8.1 ± 1.8
Nicotine	28.5 ± 1.3	25.0 ± 3.0
Saline + Stress	30.2 ± 1.6	28.0 ± 0.5
Nicotine + Stress	29.9 ± 1.5	26.8 ± 1.8

Plasma corticosterone levels were measured in rats 15 or 45 min after an injection of saline or nicotine. The results represent the means ± SEM of 6 observations. Both nicotine and stress significantly ($p<0.001$) increased plasma corticosterone over saline controls. No other differences were statistically significant.

TABLE 2

EFFECTS OF NICOTINE ON CORTICOSTERONE RECOVERY

Treatment	Plasma Corticosterone μg/100 ml
Saline Control	10 ± 1
Saline + Stress	14 ± 2
Nicotine (0.4 mg/kg) + Stress	21 ± 3*†

Plasma corticosterone levels were measured in rats which had spent 30 min on the elevated platforms followed by a 30 min recovery period. Results are the means ± SEM of 6 estimations.
*Significantly different from saline control $p<0.05$
†Significantly different from saline + stress $p<0.01$

TABLE 3

EFFECTS OF NICOTINE ON PLASMA CORTICOSTERONE AND HIPPOCAMPAL 5-HT IN METYRAPONE-TREATED RATS

Time After Nicotine Injection	Saline Corticosterone μg/100 ml	Saline 5-HT μg/g	Nicotine Corticosterone μg/100 ml	Nicotine 5-HT μg/g
45	3.7 ± 0.4	0.07 ± 0.03	8.9 ± 1.6*	0.11 ± 0.01
60	5.3 ± 1.2	0.06 ± 0.02	12.1 ± 2.1*	0.06 ± 0.01
75	6.4 ± 0.7	0.13 ± 0.01	5.4 ± 0.8	0.12 ± 0.01

Rats received injections of metyrapone (50 mg/kg) on 3 consecutive days followed by an injection (0.4 mg/kg) or saline 16 hr after the last metyrapone injection. The results represent the means ± SEM of 6 observations.
*Significantly different from saline-treated rats $p<0.05$

nicotine was administered 16 hr after the last injection of metyrapone, a small, but significant, rise in the plasma corticosterone occurred which persisted for 60 minutes (Table 3). Nicotine had no effect on the low hippocampal 5-HT levels found in the metyrapone-treated rats at any of the times studied (45, 60 and 75 min). In further experiments, in which rats were injected with saline and were placed on the platforms 16 hr after the final injection of metyrapone, a marked rise in the level of plasma corticosterone was observed. After 45 min on the platforms the levels of plasma corticosterone in these stressed rats were significantly ($p<0.001$) greater than those observed in response to nicotine in unstressed rats (Table 4). When a similar comparison was made in rats which had not received prior treatment with metyrapone, the plasma corticosterone concentrations in nicotine-treated unstressed rats and stressed rats which had received saline were both high (about 30 μg/100 ml) and were not significantly different.

DISCUSSION

The results reported here, which have shown that the administration of nicotine to unstressed rats causes an increase in plasma corticosterone levels, are in agreement with the observations of Kershbaum *et al.* [9] who summarized evidence to suggest that nicotine exerts this effect by increasing the secretion of ACTH from the pituitary gland. Nicotine did not affect the concentration of NA in either the hypothalamus or hippocampus, but did cause significant changes in the hippocampal 5-HT concentration,

TABLE 4

EFFECTS OF NICOTINE AND STRESS ON PLASMA CORTICOSTERONE IN METYRAPONE-TREATED RATS

Treatment	Plasma Corticosterone μg/100 ml
Saline	6 ± 1
Nicotine	13 ± 3*
Saline + Stress	35 ± 3†‡

Rats received injections of metyrapone (50 mg/kg) on 3 consecutive days followed by an injection of nicotine (0.4 mg/kg) or saline 16 hr after the last metyrapone injection. Some of the rats given saline were then stressed by being placed on the raised platforms. The rats were killed 45 min after receiving the nicotine or saline injections. The results represent the means ± SEM or 6 observations.

*$p<0.01$, significantly different from saline-treated rats

†$p<0.001$, significantly different from saline-treated rats

‡$p<0.001$, significantly different from nicotine-treated rats

changes which did not reflect a general response to nicotine throughout the brain since no changes were observed in hypothalamic 5-HT.

In the present investigation, the studies with nicotine were extended by examining its effects in rats subjected to psychological stress by being placed on elevated platforms. In the absence of nicotine, the stressful nature of this procedure was demonstrated by the marked elevation in plasma corticosterone levels which persisted while the rats remained on the platforms. Nicotine administration had no effect on the high plasma corticosterone concentrations found in rats placed on the elevated platforms, results which do not support the recent report [5] that nicotine can reduce plasma corticosterone levels in stressed rats. The only significant change in hippocampal 5-HT observed in rats placed on the platforms was a small increase at 15 min, whereas, if the rats received nicotine before going on the platforms, the levels were significantly reduced at 45 and 75 min. However neither these changes, or those observed in unstressed rats given nicotine, can be clearly related to changes in the plasma corticosterone levels.

Metyrapone administration significantly reduced plasma corticosterone levels in control rats and much diminished the response to nicotine whereas, by comparison, the response to stress was little affected. The acute effect of metyrapone is a specific inhibition of 11-hydroxycorticosteroid synthesis in the adrenal cortex [4, 8, 10], an effect which persists for approximately 4 hr after administration [8,17]. In these experiments, however, rats were studied at 16 to 18 hr after the last injection when, judging by the corticosterone levels in the stressed rats, the adrenals had recovered much of their capacity to synthesise corticosterone. Therefore, since the present results have shown that the metyrapone injections caused a substantial lowering of the hippocampal, but not the hypothalamic, 5-HT concentration, it seems possible that the low plasma corticosterone levels found in the control and nicotine-treated rats, following metyrapone administration, may be due to an effect on the central control of ACTH secretion rather than a direct effect on the adrenal cortex. This possibility is supported by the recent report [13] that hippocampal, but not hypothalamic, 5-HT may play a role in controlling the secretion of ACTH in unstressed rats, but that a functionally separate control mechanism, probably not involving 5-HT, operates in stressed rats [7, 14, 18]. If this is the case then the results now reported suggest that the effects which nicotine exerts on the pituitary-adrenal system may be mediated, to a substantial extent, through the control mechanism for the unstressed situation.

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