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Effects of Chronic Nicotine Administration on the Response and Adaptation to Stress

M. E. M. Benwell and D. J. K. Balfour

Department of Pharmacology and Therapeutics, The Medical School, Ninewells Hospital, Dundee DD1 9SY, Scotland

Abstract. The effects of chronic nicotine administration (0.4 mg/kg for 40 days) and its withdrawal on the adrenocortical response to acute and repeated exposure to stress have been examined and related to changes in brain 5-hydroxyindole levels. No significant effects on the response to acute stress were observed. Repeated exposure to the stressful procedure resulted in complete adaptation of the adrenocortical response and the development of a significant ($P < 0.01$) positive correlation between the plasma corticosterone and hippocampal 5-HT concentrations. In nicotine-treated rats, complete adaptation did not occur and the plasma corticosterone showed a significant ($P < 0.05$) negative correlation with hippocampal 5-HT. Nicotine withdrawal was not associated with any reduction in plasma corticosterone, but did abolish its relationship with hippocampal 5-HT.

Key words: Nicotine – Withdrawal – Plasma corticosterone – Stress – Adaptation – 5-Hydroxytryptamine – Rat

Previous studies have shown that both the acute and chronic administration of nicotine to unstressed rats causes relatively specific changes in the concentrations of 5-hydroxyindoles in the hippocampus (Balfour et al. 1975; Benwell and Balfour 1979). The acute administration of nicotine also increases plasma corticosterone concentration (Balfour et al. 1975; Turner 1975), although chronic treatment with the drug rapidly induces tolerance to this effect and, in these chronically treated animals, it is nicotine withdrawal which causes a small increase in plasma corticosterone (Benwell and Balfour 1979).

Behavioural studies suggest that nicotine dependence may be most apparent in stressful situations (Morrison 1974). However, although the performance of nicotine-dependent rats in an unsignalled Sidman avoidance schedule could be related to hippocampal 5-HT and also to pituitary-adrenocortical activity, no changes were observed in either parameter that could be attributed directly to chronic nicotine administration or its withdrawal (Balfour and Morrison 1975). Clearly, however, the behavioral training itself could have masked the effects of nicotine on hippocampal 5-HT and pituitary-adrenocortical activity and, therefore, the present study seeks to explore this possibility further by examining the effect of chronic nicotine in rats exposed to a stressful situation which is devoid of any active avoidance component.

Offprint requests to: Dr. D. J. K. Balfour

Materials and Methods

Mase Sprague-Dawley rats (Charles River), weighing approximately 150 g at the beginning of the experiment, were used. The animals, which were housed in pairs, were allowed free access to food and water and were exposed to light daily between 8 a.m. and 8 p.m.

Effects of Chronic Nicotine on the Response to Acute Psychological Stress. Rats were pretreated with nicotine (12 rats) or saline (12 rats) for 39 days using a protocol described previously (Benwell and Balfour 1979). In this protocol, each rat received on SC injection of nicotine (0.4 mg/kg) or saline on the 5 working days of each week. The nicotine was given in the form of nicotine hydrogen tartrate solution (the dose calculated in terms of the free base), which was adjusted to pH 7.0 by the addition of a small quantity of NaOH prior to injection.

On the last day of the experiment (day 40), half of the rats pretreated with nicotine were given nicotine while the remainder received saline. Immediately after these last injections, each rat was stressed for 30 min by being placed individually on an open platform which was 40 cm square with 3 cm clear Perspex sides and which was placed at the top of a 1 m pole (Balfour and Reid 1979). Half the rats which had been pretreated with saline were also exposed to stress for 30 min, while the remainder (an unstressed control group) were returned to their home cages. At the end of this period on the platforms, or after 30 min in their home cages, the rats were killed immediately by cervical dislocation. Blood samples were taken from the trunks for the estimation of plasma corticosterone, according to the method of Mattingly (1962), and the brains were removed rapidly and dissected on a chilled glass plate into the hypothalamus, hippocampus and the remainder (which was pooled together), using the procedure described by Glowinski and Iversen (1966). The concentrations of 5-HT and 5-HIAA in each brain sample were assayed using the method of Curzon and Green (1970).

Effects of Nicotine on the Response of Rats Exposed Chronically to Psychological Stress. Rats were given daily injections of nicotine (0.4 mg/kg) or saline as already described. However, after each injection, the rats were placed on the elevated platforms for 30 min before they were returned to their home cages, the only exception being a group of unstressed control animals which were returned to their home cages immediately after each injection of saline.

On the last day of the experiment, the rats were treated in exactly the same way as on the previous days with the

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Table 1. Effects of nicotine and its withdrawal on the plasma corticosterone concentration in acutely stressed rats. The rats were pretreated (39 days) with nicotine (0.4 mg/kg) or saline and then exposed to stress (30 min) following a last injection of saline (saline and withdrawn groups) or nicotine on day 40. The control group received saline throughout and were not stressed on day 40. The results are means ± SEM of six observations

Treatment group	Plasma corticosterone (μg/100 ml)
Control	12 ± 2
Saline	29 ± 3**
Nicotine	36 ± 2**
Withdrawn	30 ± 3**

Significantly different from control, ** $P < 0.01$

Table 2. Effects of nicotine and its withdrawal on adaptation to repeated stress. The rats were given saline or nicotine (0.4 mg/kg) and exposed to stress (30 min) daily for 39 days. Control received saline but were not exposed to stress. On day 40, the animals received the same treatment with the exception of the withdrawn group wich received saline in place of nicotine. The results are means ± SEM of eight observations

Treatment group	Plasma corticosterone (μg/100 ml)
Control	11 ± 2
Saline	14 ± 1
Nicotine	25 ± 4*,***
Withdrawn	26 ± 4**,***

Significantly different from control * $P < 0.05$; ** $P < 0.01$
Significantly different from saline group *** $P < 0.05$

Table 3. Correlation coefficients between plasma corticosterone and brain 5-HT in rats repeatedly exposed to stress (Spearman rank-correlation coefficent)

Treatment group	Hippocampus	Hypothalamus	Residual brain
Control	−0.393	0.336	−0.558
Saline	0.928**	0.047	0.238
Nicotine	−0.827*	0.149	−0.744*
Withdrawn	0.092	0.684	0.095

* $P < 0.05$; ** $P < 0.01$

exception of a group of rats (the nicotine-withdrawn group), which were given an injection of saline in place of the usual nicotine. The rats were killed immediately after the final 30 min session on the platforms, or in their home cages in the case of the unstressed controls, and the tissues separated and analysed as described. There were eight rats in each of the four treatment groups.

All the rats, on both experimental protocols, were killed between 9:30 − 12 noon to minimise the effects of diurnal variation on plasma corticosterone levels (Hodges 1970).

Statistical Analysis. The data were analysed using one-way analysis of variance followed by the Newman-Keuls test for differences among the means. Correlations between parameters measured in the same group of animals were assessed using the Spearman rank-correlation coefficient.

Results

The mean plasma corticosterone concentration measured in rats after a single 30-min session on the elevated platforms was significantly higher ($P < 0.01$) than the mean value for unstressed control rats (Table 1), suggesting that the procedure was stressful. Acute exposure to the procedure had no significant effects on the concentrations of 5-HT or 5-HIAA in any of the brain regions studied.

Neither the prior chronic administration of nicotine nor its withdrawal on the last day of the experiment caused any significant changes in the plasma corticosterone levels of the acutely stressed rats (Table 1), although the increase observed in the rats given nicotine on the last day approached statistical significance. The treatments also had no effects on the 5-hydroxyindole concentrations in any of the brain regions investigated.

Repeated daily 30-min sessions on the platforms resulted in adaptation to the procedure so that, after 40 sessions on the platforms, the plasma corticosterone levels in these rats were not significantly different to those found in unstressed control animals. However, if the rats were given nicotine prior to each session on the elevated platforms, the plasma corticosterone concentrations remained significantly higher ($P < 0.05$) than those measured in unstressed controls, and were also significantly higher ($P < 0.05$) than the levels measured in saline-treated rats exposed to the stress procedure for the same period of time (Table 2). Nicotine withdrawal on the last day of the experiment did not reduce the high plasma corticosterone levels observed in the nicotine-treated rats.

Adaptation to the stress procedure in the saline-treated animals was not associated with any significant changes in the concentrations of 5-HT or 5-HIAA in any of the brain regions studied. Similarly, nicotine administration and its withdrawal were without effect on these brain 5-hydroxyindole concentrations. However, in the saline-treated rats, it was found that adaptation to the stressful procedure appeared to be associated with the development of a positive non-linear relationship between the hippocampal 5-HT concentration and the concentration of corticosterone in the plasma whereas, if the rats received nicotine prior to each session on the platforms, a negative relationship developed. Statistical evaluation of the data showed that the correlations between hippocampal 5-HT and plasma corticosterone in the saline- and nicotine-treated rats were significant ($P < 0.01$ and $P < 0.05$ respectively), but that the hippocampal 5-HT concentrations in the unstressed control and nicotine-withdrawn rats did not correlate significantly with the plasma corticosterone levels (Table 3). The 5-HT concentration in the residual brain of the nicotine-treated rats also showed a significant correlation ($P < 0.05$) with plasma corticosterone, a correlation which was not observed in any of the other treatment groups.

Discussion

Previous reports have suggested that nicotine might reduce the raised plasma corticosterone levels found in stressed rats (Hall and Morrison 1973; Hall et al. 1978). The results reported here, which failed to provide any evidence for a decrease in rats exposed to either acute or repeated stress, clearly do not support this hypothesis.

The chronic administration of nicotine to unstressed rats, for the same period of time as that used in this study, does not elicit any increase in the plasma corticosterone concentration (Benwell and Balfour 1979). Therefore it seems reasonable to suggest that the increased plasma corticosterone concentrations reported here reflect a nicotine-induced change in the pituitary-adrenocortical response to stress, as a result of which the animals did not exhibit the complete adaptation to the stressful procedure observed in the saline-treated rats.

The neurochemical studies, which showed that nicotine administration markedly altered the relationship between hippocampal 5-HT and plasma corticosterone, also suggest that the two groups of rats adapted to repeated stress in a different way. The effect on hippocampal 5-HT could reflect a nicotine-induced impairment of the process by which the rats remembered their previous experience of the stressful procedure and thus failed to adapt to it. This, however, seems unlikely since previous studies (Morrison 1974) indicate that nicotine does not adversely affect memory processes in stressed rats and, indeed, there is evidence to suggest that it enhances memory retention (Izquierdo and Elisabetsky 1978).

It is also possible that the changes in hippocampal 5-HT could be associated with effects on the central control of pituitary-adrenocortical activity. Nicotine is reported to protect rats from the behaviourally disruptive effects of stress, an effect which has been associated with changes in the electroencephalographic pattern of the hippocampus (Nelsen 1978). However, previous studies (Scapagnini and Preziosi 1972; Balfour et al. 1975) have failed to show any relationship between hippocampal 5-HT and plasma corticosterone levels in rats exposed to acute stress, although Gray (1981) has suggested that anti-anxiety drugs, which have been shown to reduce plasma corticosterone levels in stressed rats (Keim and Sigg 1977; Le Fur et al. 1979), exert their anxiolytic effect by reducing the flow of noradrenergic ans serotonergic impulses to the hippocampus. In the present study, the saline-treated rats which showed the greatest adaptation (i.e. those with the lowest plasma corticosterone concentrations) also had the lowest hippocampal 5-HT. One possible explanation of the results, therefore, is that habituation to the stressful procedure involves activation of an endogenous anti-anxiety system, present in the brain, whose effects on hippocampal 5-HT and adrenocortical activity are very similar to those seen in response to anxiolytic drugs.

At the present time there is insufficient data to explain the inverted relationship between hippocampal 5-HT and plasma corticosterone observed in the nicotine-treated rats, although it has been associated tentatively with the development of nicotine dependence in stressful situations (Balfour 1982). This conclusion is consistent with the fact that nicotine withdrawal, which in stressful situations causes behavioural disruption (Morrison 1974; Nelsen 1978), abolished the relationship. Clearly, however, further studies are necessary to establish this conclusion with certainty.

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