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Effect of Prolonged Exposure to Nicotine and Stress on the Pituitary-Adrenocortical Response; the Possibility of Cross-Adaptation

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CAM, G. R. AND J. R. BASSETT. *Effect of prolonged exposure to nicotine and stress on the pituitary-adrenocortical response; the possibility of cross adaptation.* PHARMACOL BIOCHEM BEHAV 20(2) 221–226, 1984.—Daily IP injections of nicotine (200 μg/kg body weight) resulted in an adaptation of the nicotine induced rise in plasma corticosterone. By 30 days the plasma corticosterone rise was not significantly different from that seen in control animals receiving an injection of saline. A similar adaptation to the plasma corticosterone response to the stress of signalled, irregular footshock was also observed. However, in the case of the exposure to stress, while the corticosterone response at day 40 was significantly less than the response seen on day 1, it was still significantly greater than the plasma corticosterone level from unstressed control animals. Cross-adaptation experiments were conducted in which animals were adapted to the steroidogenic action of nicotine and then subjected to a novel exposure to footshock stress, and vice versa. In both situations the animals responded to the novel stimulus, either stress or nicotine, with a significant rise in plasma corticosterone. It was postulated that nicotine and psychological stress act upon the hypothalamo-pituitary-adrenal axis via functionally separate pathways at the level of the corticotrophin releasing factor neuron. The separate pathways appear to differ in their ability to be inhibited by corticosterone feedback.

Nicotine Stress Corticosterone Adaptation Cross-adaptation

BEHAVIOURAL tolerance to the long-term administration of nicotine has been demonstrated in studies involving spontaneous activity [1,30], Y and T maze discrimination, and behavioural arousal [14,15].

A similar tolerance or adaptation to the action of nicotine has been shown for parameters other than behaviour. The initial increase in urinary catecholamines seen after injection of nicotine returned to control levels following 10 days administration [33,34], while the nicotine induced increased activity in EEG pattern, disappeared after six daily doses of nicotine [14,15].

Certain physiological reponses, however, provide less definite evidence for concluding tolerance or adaptation to the effects of nicotine administered for prolonged periods. For example, although a tolerance to the pressor effects of nicotine developed quickly, rendering the animal subsensitive to noradrenaline, the administration of nicotine for periods up to 8 weeks produced a supersensitive pressor response to noradrenaline [32].

The acute administration of nicotine has been shown to result in a rise in plasma glucocorticoid level, a response mediated through the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland [6]. However, as yet there have been no direct studies to show if an adaptation of the glucocorticoid response to nicotine occurs with prolonged exposure. Exposure to psychological stress is known to be associated with a rise in plasma glucocorticoids; again mediated via the release of ACTH [19]. In the case of stress the glucocorticoid response does adapt with prolonged exposure to the stressor [2,5].

The questions raised in this study, then, are (1) does adaptation of the glucocorticoid response to nicotine develop after prolonged exposure to the alkaloid; and if so (2) since both nicotine and stress exert their steroidogenic action via the release of ACTH from the pituitary gland, is the mechanism for such an adaptation common to both situations. If the mechanisms for adaptation are common to both, then cross-adaptation should occur between the glucocorticoid response to stress and the response to nicotine. Such findings may help explain the supposed anti-anxiety component of prolonged cigarette smoking [11].

METHOD

Animals

Male CSF rats, 85±5 days old at the commencement of the experiment were used in this study. The animals were housed in groups of 3 under conditions of constant tempera-

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ture and humidity (21±0.5°C; 46% humidity and subjected to a 12 hr reversed night-day schedule (light 2000 to 0800 hr) beginning at least 14 days prior to the commencement of experimentation. Food and water were provided ad lib.

Corticosterone Assay

Corticosterone was assayed by the competitive protein binding assayed as described by Murphy [20]. Corticosterone was extracted from 100 μl plasma with dichloromethane. Lypholysed human serum (Q-Pak Hyland control serum, Travenol Laboratories) was used as binding protein. Unbound 1, 2, ^{3}H-corticosterone (New England Nuclear) was removed using Florisil. The sensitivity of the assay was 2 μg/100 ml and the inter and intra assay variability was 4.6 and 4.0% respectively.

Nicotine Assay

Plasma nicotine concentrations were determined by a gas-liquid chromatographic method modified from Isaac and Rand [16]. Nicotine was extracted from the plasma into diethyl ether and was then concentrated by evaporating the solvent to a final volume of 200–500 μl. Hydrochloric acid (2 M; 100 μl) was then added and the tubes vortexed. The ether layer was discarded by aspiration. The aqueous phase was alkalinized with NaOH and 50 μl n-heptane added. Following vortexing the aqueous phase was removed and an aliquot of the heptane layer injected into a Tracor 560 gas chromatograph fitted with a nitrogen-phosphorous detector. Peak height ratios for nicotine and the internal standard quinoline were measured. Nicotine standard curves were prepared by plotting quinoline/nicotine peak height ratios against known nicotine concentrations, a linear response being observed over the range 1–150 ng. The sensitivity of the method was 0.5 ng/ml. The chromatograph columns were of glass tubing 1.85 m in length and 2 mm in diameter, packed with 3% silicone OV-17 (Alltech Associates) on Gas Chrom Q 100-120 mesh (T.M. Applied Science Laboratories). Optimum operating conditions were: injection block temperature 200°C; column oven temperature 150°C; detector oven temperature 250°C; carrier gas, helium flow rate 25 ml/min; hydrogen flow rate 2.5 ml/min; air flow rate 125 ml/min. Under these conditions the retention times for nicotine and quinoline were 2.2 min and 3.0 min respectively.

Stress Apparatus

Animals were placed in automated one-way avoidance boxes (Lafayette Model No. 85200). An escape platform was made available to the animal by an automated movable partition. A light conditioned stimulus (CS) of 2 W was located on the wall of the grid opposite to the escape platform. The unconditioned stimulus (UCS) was delivered by a generator-scrambler through the grids as a 2 mA, 50 pulse/sec square wave. Each rat was placed on the escape platform at the commencement of the treatment session. On each trial the CS onset 4 sec before the animal was pushed by the movable partition from the platform onto the grid which was simultaneous with the onset of the UCS. At this point the movable partition immediately retracted and the animal was able to jump from the grid to the re-exposed platform with a minimum latency of 0.3 sec. The UCS was terminated by the return of the animal to the platform. The stress treatment consisted of 7 CS-UCS exposures randomly placed in a 35 min stress session. Stress treatments were carried out between the hours of 0900–1200.

Procedures

Prolonged nicotine administration on the plasma corticosterone response. Animals received daily intraperitoneal injections of 200 μg nicotine hydrogen tartrate/kg body weight for periods of 1, 5, 10, 15, 20, 30, 45 and 60 days. Control animals received injections of saline. In both cases injection volumes corresponded to 0.1 ml/100 g body wt. Each time point consisted of 12 nicotine and 12 control animals. All animals were killed 25 min after their last injection of nicotine or saline. The animals were killed by cervical dislocation and exsanguinated. The blood was collected in heparinized tubes and centrifuged to obtain cell free plasma which was then frozen. The 200 μg/kg dose of nicotine and the 25 min killing time were chosen since it had been previously shown that this dose produced a substantial rise in plasma corticosterone with a peak occurring at approximately 20 to 25 min [6]. Corticosterone levels in plasma were assayed as described above.

Plasma nicotine concentrations following prolonged administration. Animals received daily injections of 200 μg nicotine/kg over the same time period as described above. Animals were killed in groups of 8 at either 10 min or 25 min following their last injection of nicotine. Plasma samples were collected as before and assayed for nicotine. The two times of killing after the last injection of nicotine (10 and 25 min) were chosen since they represent the peak in plasma nicotine concentration and the secondary plateau in the decay phase [6].

Long-term stress on plasma corticosterone response. Animals were subjected to a stress session once daily for periods of 1, 10, 40 and 60 days. For each time period the stress and control (unstressed) groups consisted of 6 animals. Immediately following the completion of the last stress session the animals were sacrificed as described previously and the plasma corticosterone levels determined by the competitive protein binding assay.

Cross-adaptation study. Animals were divided into 3 sets of groups. In the first set animals were either acutely exposed to nicotine or stress. Animals were given an IP injection of 200 μg/kg nicotine (n=12) or saline (n=10) and sacrificed 25 min later (the peak of the corticosterone response to nicotine) and plasma samples obtained. A second group of animals were subjected to irregular signalled footshock (n=12) for 35 min (the time required to give peak corticosterone response to this stressor) and then sacrificed. Control animals (n=10) were removed from their home cage and immediately sacrificed. Plasma samples were obtained as described previously.

In the second set animals were chronically exposed to either nicotine or stress. The first group of animals received daily injections of either 200 μg/kg nicotine (n=12) or saline (n=10) for a period of 45 days. These animals were sacrificed 25 min after their last injection and plasma samples obtained. A second group of animals (n=12) were subjected to a 35 min daily stress session for 45 consecutive days. These animals were sacrificed immediately following the completion of their last stress session. Control animals (n=10) were removed from their home cage immediately prior to sacrifice and had not been subjected to any previous stress episode. Plasma samples were obtained as before.

The third set of animals were chronically exposed to

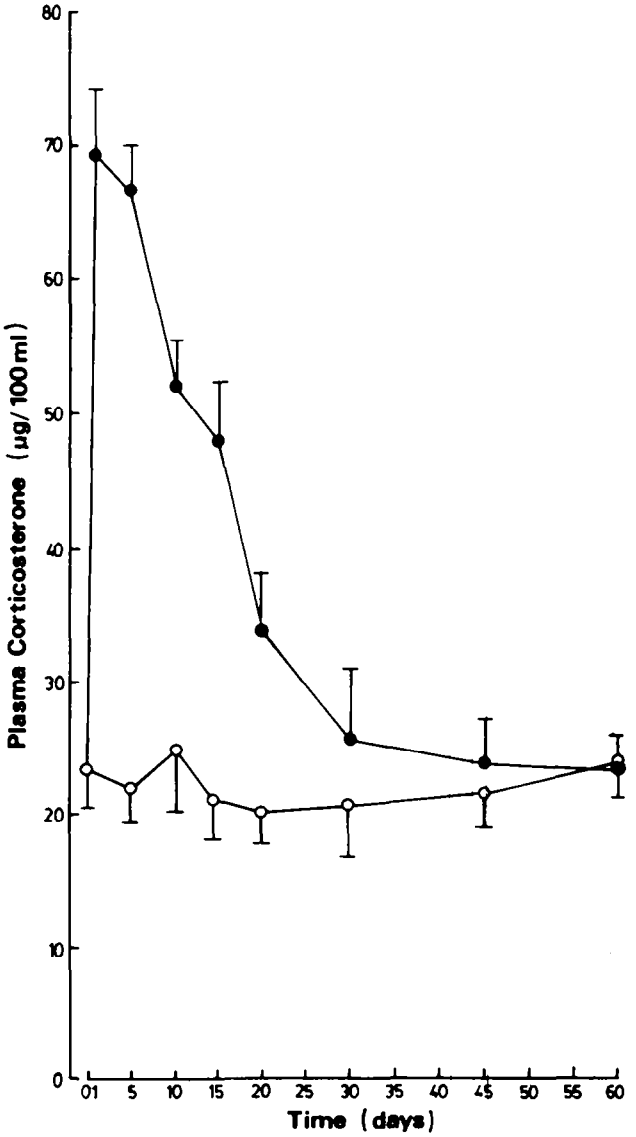

FIG. 1. Plasma corticosterone levels following daily IP injection of 200 μg/kg nicotine hydrogen tartrate. Each point represents the mean level of plasma steroid for 12 animals. Vertical bars denote ±S.E. Open circles represent control animals receiving saline injections, closed circles represent nicotine injected animals.

either stress or nicotine, then acutely exposed to the alternative stimulus. One group of animals received daily injections of 200 μg/kg nicotine (n=12) for a period of 44 days. On day 45 these animals were subjected to irregular signalled footshock for a period of 35 min, after which they were immediately sacrificed and plasma samples obtained. A second group of animals (n=12) were subjected to a 35 min daily stress session for 44 days. On day 45 these animals were given an IP injection of 200 μg/kg nicotine and sacrificed 25 min later. Plasma samples were obtained.

All plasma samples were assayed for corticosterone concentration.

TABLE 1

MEAN PLASMA NICOTINE LEVELS (±S.E.) FOLLOWING THE DAILY IP INJECTION OF 200 μg/kg NICOTINE

Time (days)	Plasma Nicotine (ng/ml) 10 min	25 min
1	48.6 ± 5.9	19.1 ± 3.4
5	46.7 ± 3.6	22.3 ± 5.9
10	47.1 ± 4.1	16.3 ± 8.1
15	43.3 ± 6.7	17.4 ± 4.6
20	44.9 ± 1.8	20.2 ± 1.7
30	43.7 ± 3.8	17.2 ± 2.1
45	42.8 ± 3.0	18.9 ± 3.6
60	43.5 ± 2.5	16.8 ± 7.4

Plasma sample taken either 10 min or 25 min following injection. Number of animals/group=8.

RESULTS

Prolonged nicotine administration on plasma corticosterone response. The results of this experiment are shown in Fig. 1. A one-way analysis of variance showed a significant variation in nicotine induced plasma corticosterone over the time period studied, $F(7,77)=18.2$, $p<0.05$. The critical values for a significant difference between any two means using the Tukey test are 9.7 μg/100 ml at the 5% level and 11.2 μg/100 ml at the 1% level. By day 30 the corticosterone level had returned to control values. A one-way analysis of variance showed no significant difference between the nicotine induced plasma corticosterone levels at 30, 45 and 60 days, $F(2,33)=0.59$, $p>0.05$. The plasma corticosterone levels at 30, 45 and 60 days were not significantly different from their corresponding control values (unpaired "t" test, $p>0.05$ in all cases).

Plasma nicotine concentrations following prolonged administration. The results for this experiment are shown in Table 1. No significant reduction in plasma nicotine concentration, either at the peak level or secondary plateau level, was found over the time period studied (a one-way analysis of variance; $p>0.05$ in both cases). The mean plasma nicotine levels ranged from 42.8 to 48.6 ng/ml for the 10 min group and 16.5 to 22.3 ng/ml for the 25 min group. These values agree well with the values reported previously [6] for plasma levels of nicotine following an IP injection of the same dose of nicotine; 42.5 ng/ml at 10 min and 17.5 ng/ml at 25 min.

Long-term stress on plasma corticosterone response. The results of this experiment are shown in Table 2. At all time periods investigated exposure to the stress resulted in a significant increase in plasma corticosterone (unpaired "t" test, $p<0.001$ in all cases). No significant difference was observed in the values obtained at 1 and 10 days ($p>0.05$) nor were the values at 40 and 60 days significantly different from one another ($p>0.05$). However there was a significant difference between the values, obtained at day 10 and compared with those obtained at day 40 ($p<0.001$). The results obtained in this study are in close agreement with those reported previously for this stressor [2,5].

Cross-adaptation study. The results of this study are shown in Fig. 2. A novel injection of 200 μg/kg nicotine or

TABLE 2

MEAN PLASMA CORTICOSTERONE LEVELS (±S.E.) OF RATS SUBJECTED TO STRESS DAILY

Days of stress	Corticosterone (μg/100 ml of plasma) Control	Stressed	Unpaired t test $p<$
1	19.8 ± 3.4	88.2 ± 4.8	0.001
10	23.1 ± 4.8	81.3 ± 5.4	0.001
40	20.0 ± 3.0	42.0 ± 3.3	0.001
60	20.8 ± 3.1	40.9 ± 3.9	0.001

Number of animals/group=6.

the novel exposure to the stress apparatus on day 1 resulted in an plasma corticosterone rise to 72 μg and 95 μg/100 ml plasma respectively. These values are in close agreement with those obtained for similar procedures reported earlier. By day 45 of continued exposure to either nicotine or stress the values had fallen to 30 μg/100 ml and 44 μg/100 ml respectively. In the case of the chronic nicotine study the plasma corticosterone level was not significantly different from the corresponding control value (unpaired "t" test, $p>0.05$). The response to chronic stress, however, while significantly reduced compared to day 1 was still significantly greater than the corresponding control group (unpaired "t" test, $p<0.05$).

Following the adaptation of the corticosterone response to chronic nicotine administration, acute exposure to the stress apparatus resulted in a rise in plasma corticosterone to 91 μg/100 ml plasma. This value was not significantly different from the value obtained for day 1 of the stressor alone. (Unpaired "t" test, $p>0.05$) but was significantly different from that of animals chronically exposed to either stress or nicotine. In the case of chronic exposure to footshock stress followed by an acute exposure to nicotine, the nicotine injection resulted in the plasma corticosterone level rising to 65 μg/100 ml plasma. This value was not significantly different (unpaired "t" test; $p>0.05$) from that obtained for the novel injection of nicotine on day 1, but once again was significantly greater ($p<0.05$) than either the chronic nicotine or stress treatments at day 45.

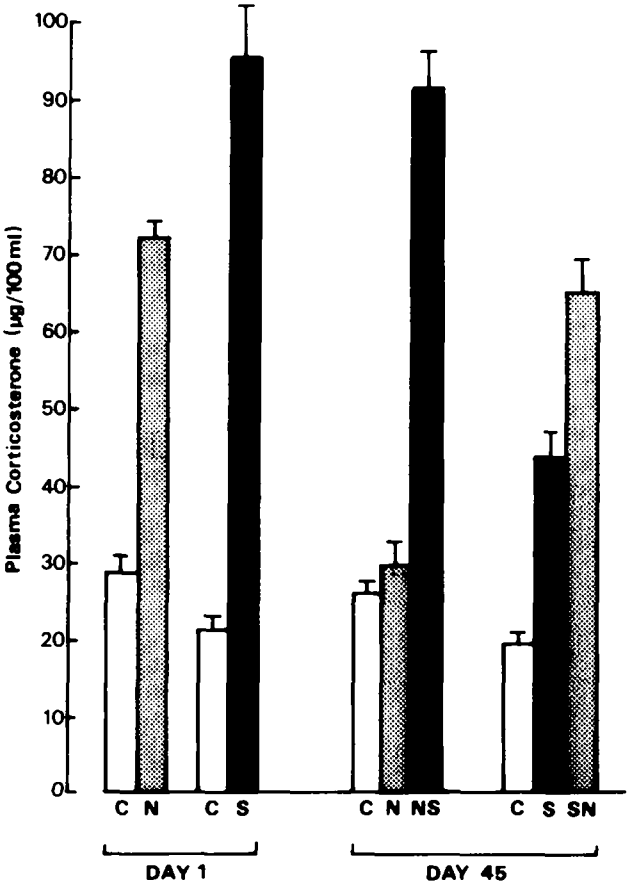

FIG. 2. Plasma corticosterone levels after acute (1 day) and prolonged (45 days) exposure to either 200 μg/kg nicotine hydrogen tartrate (N) or footshock stress (S). The NS group (n=12) received IP injections of nicotine for 44 days followed by acute exposure to footshock stress on day 45. The SN group (n=12) was stressed for 44 days and received a nicotine injection on day 45. The control groups (C) for the nicotine experiments received saline injections for either 1 day or 45 days. For the stress experiments control animals were sacrificed immediately after removal from their home cage. Each column represents the mean of 12 animals for N and S and 10 animals for C. Vertical bars denotes±S.E.

DISCUSSION

Prolonged nicotine administration resulted in the adaptation of the nicotine induced plasma corticosterone rise. The magnitude of the nicotine elicited corticosterone response declined over the time period studied, returning to control levels by 30 days. This finding supports the human studies on cigarette smoking. The introduction of cigarette smoking to inexperienced smokers [13] or to chronic smokers who had abstained from smoking for at least 12 hr [18] resulted in an increased level of plasma glucocorticoids. However, habitual moderate smoking was not associated with any increase in pituitary-adrenocortical activity [28]. The diminished plasma corticosterone response with prolonged nicotine administration was not a function of any change in the rate of metabolism of nicotine since the plasma nicotine concentrations remained unchanged throughout the time period studied. The metabolism of nicotine has been reported previously not to change significantly following chronic administration of the drug [29,31].

Adaptation of the pituitary-adrenal cortical axis with continuing exposure to an aversive stimulus has been reported for a number of different stressors. Bassett and Cairncross [2] reported that daily exposure to unpredictable footshocks, a stressor associated with a large psychological component [4], resulted in an adaptation of the stress induced plasma corticosterone rise. The initial elevation remaining unchanged for the first 5 days exposure to the stressor before gradually falling to re-establish a new plateau level by day 40. This new level, which was significantly less than the initial extreme level, but significantly greater than the unstressed control level, was then maintained to at least 60 days. While the corticosteroid response to the stressor was greatly reduced following chronic exposure, it was not completely lost. This observation was later confirmed by Bassett

and Pollard [5] using the same stress regime and by the present studies. Prolonged exposure to irregular signalled footshock for 40 days did not result in a complete adaptation of the corticosterone response. The plasma corticosterone levels being reduced but still significantly greater than those of the control unstressed animals. In this regard the adaptation profiles for the corticosterone response to nicotine and stress differ. In the case of nicotine administration the corticosterone response was completely lost by day 30. This difference may imply dissimilar long-term adaptive mechanisms operating on the pituitary-adrenal axis in the case of nicotine and psychological stress.

A variety of mechanisms have been suggested for the adaptation of the plasma corticosterone response following chronic exposure to a stressor. These include (1) inhibition of the hypothalamo-pituitary-adrenal axis by a negative feedback mechanism [9,17], or by central inhibition mechanisms; (2) an inability of the system to respond either at the pituitary or adrenal level [22]; or (3) an increased turnover of corticosteroids peripherally [21]. Sakellaris and Vernikos-Danellis [23] suggested that the decrease in plasma corticosteroid response to a stressful stimulus following chronic exposure was not due to a refractory state of the adrenal or the pituitary nor to a tonic inhibitory mechanism since a rapid and significant response did occur when the animals were exposed to a second type of stressor. However, the adapted animals did not seem to possess a normal pituitary-adrenal axis since they showed a greater sensitivity and faster response onset to the second stimuli when compared with controls [10,23]. It has been proposed that repeated chronic stress may cause an increased drive in the ACTH secreting mechanisms which compensate for or override the corticosteroid feed-back mechanism [7,8].

The cross-adaptation experiments reported in the present study indicate that the habituated pituitary-adrenal axis was capable of responding to a novel stimulus. Although the response to either the injection to nicotine or exposure to the stressor had adapted following chronic exposure, the axis was able to respond to a novel stimulus without any appreciable change in the amplitude of the plasma corticosterone response. An enhanced response to the novel stimulus in previously adapted animals, when compared to the novel stimulus alone, was not observed in this experiment; unlike the results of Sakellaris and Vernikos-Danellis [23].

It would appear that nicotine and psychological stress may act upon the hypothalamo-pituitary adrenal system via functionally separate pathways. The adaptation curves for both nicotine and stress are different and there is little if any cross-adaptation. The adapted glucocorticoid response cannot represent a self induced increase in hepatic catabolism of corticosterone, as suggested by Orrenius *et al.* [21] since a novel exposure to the second stimulus can still produce a corticosterone response in an otherwise adapted animal. For a similar reason a suppressed ACTH release through a general feedback inhibition from the elevated circulating levels of corticosterone cannot apply. Under certain circumstances pituitary-adrenal function may lack precise negative feedback control since large doses of corticosteroids or elevated endogenous levels also fail to block completely the release of ACTH in response to acute stress [7, 12, 27].

It is possible that the functionally different pathways may be at the level of the neuron concerned with release of releasing factor. It has been suggested that there may be at least two neural pathways to the CRF neurone; a low threshold pathway which can be inhibited by chronically elevated levels of corticosteroids, and a high threshold pathway which appears to be steroid resistant [7,17]. Certain stresses are capable of eliciting an apparently normal pattern of CRF responses even during maximally effective corticosteroid blockade of the hypothalamus [7]. The highly dexamethasone-sensitive stimulus of sham adrenalectomy and the steroid resistant stimulus of laparotomy-intestinal traction both produce similar elevations in hypothalamic CRF, circulating ACTH and corticosterone [24]. If nicotine and stress are acting upon such functionally separate pathways then the nicotine pathway would appear to be more steroid sensitive than the pathway associated with the stress response. The corticosterone response to nicotine adapted completely whereas the stress response only partially adapted.

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