ELSEVIER

European Journal of Pharmacology 285 (1995) 317–321

ejp

Short communication

Intermittent cocaine exposure causes delayed and long-lasting sensitization of cocaine-induced ACTH secretion in rats

E. Donné Schmidt *, Fred J.H. Tilders, Anton W.J.W. Janszen, R. Binnekade, Taco J. De Vries, Anton N.M. Schoffelmeer

Graduate School Neurosciences Amsterdam, Research Institute Neurosciences Vrije Universiteit, Faculty of Medicine, Department of Pharmacology, Van der Boechorststraat 7, 1081 BT Amsterdam, Netherlands

Received 13 July 1995; revised 17 August 1995; accepted 22 August 1995

Abstract

In view of the possible role of the hypothalamus-pituitary-adrenal axis in the long-term effects of drugs of abuse, we studied the response of the hypothalamus-pituitary-adrenal axis to cocaine challenges 3 and 14 days after cocaine withdrawal. Three days after intermittent cocaine exposure, the cocaine-induced increase of plasma adrenocorticotropic hormone (ACTH) is unchanged, whereas after 14 days the ACTH response is enhanced 2-fold. The cocaine-induced increase of plasma corticosterone is enhanced approximately 1.5-fold both 3 and 14 days after cocaine withdrawal. Apparently, prior cocaine treatment causes a delayed sensitization of cocaine-induced ACTH secretion and long-lasting corticosterone hyper-responsiveness. We propose that the long-lasting changes in the hypothalamus-pituitary-adrenal axis may facilitate drug-induced long-term behavioral sensitization.

Keywords: ACTH (adrenocorticotropic hormone); Cocaine; Corticosterone; Hypothalamus-pituitary-adrenal axis

1. Introduction

Corticotropin releasing hormone (CRH) neurons that control the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland have their secretory terminals in the external zone of the median eminence. These neurons can produce, store and release vasopressin, a neuropeptide that strongly potentiates the ACTH releasing effect of CRH (Tilders et al., 1993). Recently, we reported that various stressors (e.g. interleukin-1, electric footshocks) that activate these CRH neurons, cause a delayed increase of the vasopressin stores in the CRH terminals in the external zone of the median eminence 1–3 weeks later (Tilders et al., 1993; Van Dijken et al., 1993; Schmidt et al., 1995) and enhanced ACTH and corticosterone responses when tested 2 weeks later (stress sensitization) (Van Dijken et al., 1993; Schmidt et al., 1995). Psychostimulant drugs also activate hypothalamic CRH neurons controlling ACTH secretion (Rivier and Vale, 1987; Rivier and Lee, 1994). Intermittent exposure to drugs of abuse is well known to cause long-lasting sensitization towards the psychomotor effects of these drugs, which is proposed to play a role in the vulnerability towards the acquisition of drug dependence (Piazza et al., 1990; Robinson and Berridge, 1993). Because it has been suggested that adrenal corticosteroids facilitate the development of dependency to drugs of abuse (Piazza et al., 1991, 1994) it is of interest to determine whether cocaine exposure causes hyper-responsiveness of the hypothalamus-pituitary-adrenal axis similar to that seen after exposure to stressors. Although it has been reported that repeated exposure to cocaine does not induce sensitization of ACTH or corticosterone responses, these studies were performed shortly after or during drug exposure (Borowsky and Kuhn, 1991; Levy et al., 1992; Torres and Rivier, 1992). In view of our recent studies showing that stress-induced changes in the hypothalamus-pituitary-adrenal axis become manifest with a delay of several days, we investigated whether intermittent cocaine may also cause delayed and enduring changes in the hypothalamus-pituitary-adrenal axis.

* Corresponding author. Tel.: (31) (20) 4448107; fax: (31) (20) 4448100; e-mail: ed.schmidt.pharm@med.vu.nl.

0014-2999/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0014-2999(95)00540-4

2. Materials and methods

2.1. Animals and experiments

Male Wistar rats (Harlan, CPB, Zeist, Netherlands), 160–180 g body weight upon arrival, were housed 2 per cage under controlled conditions (light period from 7.00 a.m. to 7.00 p.m.) at 22 ± 2°C, for 7 days prior to the beginning of the experiments. Food and water were available ad libitum. Animals were subjected to (non-invasive) handling twice daily during 3 days prior to exposure to i.p. injection with either cocaine (15 mg/kg) or saline (0.5 ml, pyrogen free). To establish the kinetics of acute plasma ACTH and corticosterone responses, groups of rats ($n = 6$–8) were given cocaine (15 mg/kg i.p.) or saline and were decapitated 0–30 min later. Other groups of rats received either no injection, 5 once-daily injections of cocaine (15 mg/kg) or vehicle. Three or 14 days later, groups of these rats ($n = 8$) were given either no additional treatment, or injections of saline or cocaine (15 mg/kg i.p.) and were killed by decapitation 20 min later (between 10.00 and 11.00 a.m.). Trunk blood was collected in ice-cold heparin-coated tubes and centrifuged (1000 × g, 15 min, 4°C). Aliquots of plasma were stored at −20°C until assayed.

2.2. Determination of ACTH, corticosterone and prolactin

ACTH concentrations were measured by a radioimmunoassay (Van Oers et al., 1992) using a specific antiserum (8514) directed against the midportion of ACTH, kindly provided by Dr G.B. Makara (Budapest, Hungary). Synthetic rat ACTH (Peninsula, Belmont, CA, USA) was used as a standard. The sensitivity of the assay was 10 pg/ml plasma (0.5 pg/tube). The intra- and inter-assay variations were 4% and 7% respectively.

Corticosterone concentrations were determined (Van Oers et al., 1992) by using antiserum S-5230676, kindly supplied by Dr T.J. Benraad (Nijmegen, Netherlands). Corticosterone (Sigma, St. Louis, MO, USA) was used as a standard. The sensitivity was 0.3 ng/ml plasma (12 pg/tube). The intra- and inter-assay variations were 4.7% and 5.4% respectively.

Plasma prolactin concentrations were determined using reagents kindly supplied by NIADDK and are expressed in ng RP3 per ml plasma. The intra- and inter-assay variations were 5% and 8% respectively.

2.3. Statistics

ACTH and corticosterone data were analyzed by analysis of variance (ANOVA) followed by the Fisher least-significant difference test, using the NCSS statis-

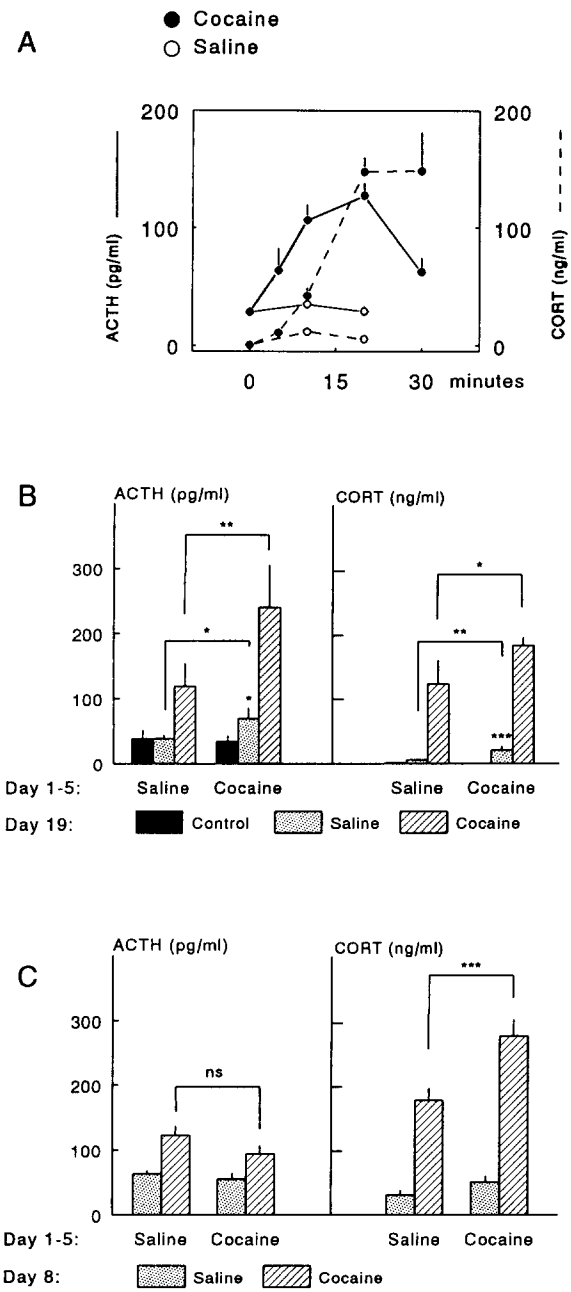

Fig. 1. Acute and long-term effects of cocaine administration on ACTH and corticosterone responses. A: Acute ACTH (solid lines) and corticosterone (CORT, broken lines) responses to cocaine (15 mg/kg i.p., solid circles) or to saline (0.5 ml, open circles). Groups of rats were killed at time-intervals of 0–30 min after injection. Data are presented as mean and S.E.M. ($n = 6$). B: Effect of cocaine pretreatment on cocaine-induced ACTH and corticosterone (CORT) responses 14 days later. Rats pretreated (days 1–5) with saline or cocaine were exposed (day 19) to nothing (controls, black bars = basal levels), saline (dotted bars) or cocaine (15 mg/kg, hatched bars) and killed 20 min later. Data are presented as means and S.E.M. ($n = 8$). C: As in B rats were pretreated with saline or cocaine but challenged with saline or cocaine 3 days (day 8) instead of 14 days after the last injection. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant.

tical software program (Kaysville, UT, USA). ANOVA was preceded by tests for homogeneity of variance and for normality of residuals. Using the same software, the prolactin data were analyzed by the nonparametric Kruskall and Wallis test, because the preceding tests revealed that the data did not fulfil the assumptions of ANOVA and because some values of the prolactin data were below detection limits of the analysis (< 0.1 ng/ml).

3. Results

3.1. Acute ACTH, corticosterone and prolactin responses

Cocaine (i.p.) evokes a fast rise of both ACTH and corticosterone levels. ACTH peaks at 20 min at 127.4 ± 10.6 pg/ml whereas corticosterone appears to reach a plateau (147.7 ± 12.1 ng/ml) at approximately 20 min after cocaine injection (Fig. 1A). Saline injection (controls) did not evoke significant rises in plasma ACTH or corticosterone levels. Prolactin levels decreased from 1.93 ± 0.62 ($t = 0$) to 0.33 ± 0.09 ng/ml ($P < 0.05$) at 5 min post-injection, remaining low at 10 min (0.25 ± 0.06 ng/ml, $P < 0.01$) and 20 min post-injection (0.90 ± 0.32 ng/ml, $P < 0.05$).

3.2. Long-term effects on ACTH and corticosterone responses

In order to determine possible long-term effects of cocaine on the responsiveness of the hypothalamus-pituitary-adrenal axis to later cocaine exposure, rats were treated with cocaine or saline, and were either not treated or challenged with vehicle or cocaine 14 days later. Plasma concentrations of ACTH and corticosterone were measured 20 min after the challenge (Fig. 1B), i.e. when the responses to cocaine reach near-maximal levels (see Fig. 1A). Plasma ACTH and corticosterone levels in untreated, unchallenged controls (data not shown) were not different from those in saline-pretreated control rats (38.7 ± 12.5 pg/ml ACTH and 1.4 ± 0.3 ng/ml corticosterone, Fig. 1B). Cocaine pretreatment does not affect the basal ACTH levels as determined 14 days later, but the basal levels of corticosterone (0.4 ± 0.1 ng/ml) are lower than those found in saline-pretreated or untreated controls ($P < 0.01$). A saline challenge in saline-pretreated rats does not affect the plasma levels of ACTH or corticosterone as compared to those of unchallenged saline-pretreated controls. However, a saline challenge in cocaine-pretreated rats increases the ACTH ($P < 0.05$) and corticosterone levels ($P < 0.001$) as compared to those in unchallenged cocaine-pretreated controls. A cocaine challenge in saline-pretreated rats elevates plasma ACTH and corticosterone levels to 118.7 ± 35.4 pg/ml and 122.7 ± 36.6 ng/ml corticosterone respectively. In cocaine-pretreated rats cocaine challenge induced exaggerated ACTH ($P < 0.01$) and corticosterone ($P < 0.05$) responses of 240.3 ± 64.4 pg/ml and 181.4 ± 13.0 ng/ml respectively.

In order to establish whether the cocaine-induced hyper-responsiveness of the hypothalamus-pituitary-adrenal axis occurs with a delay, we also determined the ACTH and corticosterone responses to cocaine or saline challenge 3 days after cocaine or saline withdrawal. The ACTH and corticosterone levels of cocaine-challenged rats are increased in both saline- and cocaine-pretreated groups ($P < 0.01$ vs. saline-challenged rats, Fig. 1C). In contrast to our observations 14 days after pretreatment, a cocaine challenge did not induce higher plasma ACTH levels as compared to those in saline-pretreated rats. Nonetheless, the cocaine-induced corticosterone levels (276.4 ± 25.2 ng/ml) in the cocaine-pretreated group are 1.5-fold higher than those in the saline-pretreated group (176.6 ± 18.5 ng/ml, $P < 0.001$).

3.3. Long-term effects on prolactin responses

Three days after exposure to cocaine or saline the plasma prolactin levels in saline-challenged rats do not

Table 1
Effect of cocaine pretreatment on cocaine-induced prolactin responses 3 and 14 days later

Group	Pretreatment	Challenge	Days after cocaine withdrawal			
			3 days	$P < 0.05$ vs.	14 days	$P < 0.05$ vs.
1	Saline	Control	–		1.7 ± 0.4	(3, 4)
2	Saline	Saline	3.3 ± 0.6	(3)	2.2 ± 0.5	(3, 5)
3	Saline	Cocaine	1.1 ± 0.3	(2)	0.7 ± 0.2	(1, 2, 6)
4	Cocaine	Control	–		0.7 ± 0.2	(1, 5, 6)
5	Cocaine	Saline	2.9 ± 0.8	(6)	3.8 ± 0.8	(2, 6)
6	Cocaine	Cocaine	1.1 ± 0.5	(5)	3.0 ± 1.3	(3, 4)

Three or 14 days after once-daily treatment (5 days) with saline or cocaine rats were either not challenged or challenged with saline or vehicle and decapitated 20 min later. Data represent mean ± S.E.M. Differences ($P < 0.05$) between groups 1–6 are denoted by the group numbers placed between brackets.

differ (Table 1). A cocaine challenge reduced prolactin levels in saline- ($P < 0.01$) and cocaine-pretreated ($P < 0.05$) rats which is in accordance with observations of others (Borowsky and Kuhn, 1991; Levy et al., 1992).

Fourteen days after cocaine withdrawal basal prolactin levels have decreased ($P < 0.01$) as compared to those of saline-pretreated rats. A saline challenge in cocaine-pretreated rats results in a higher ($P < 0.05$) plasma prolactin level (3.8 ± 0.8 ng/ml) than that induced in saline-pretreated rats (2.2 ± 0.5 ng/ml). Similarly, a cocaine challenge in cocaine-pretreated rats induces elevated ($P < 0.01$) plasma prolactin levels (3.0 ± 1.3) as compared to decreased levels induced in saline-pretreated rats (0.7 ± 0.2 ng/ml).

4. Discussion

In the present paper we demonstrate that exposure of adult rats to cocaine for 5 days induces a delayed and long-lasting (at least 2 weeks) increase of the ACTH response to later cocaine administration. Cocaine-induced sensitization of the ACTH response was not evident 3 days after cocaine withdrawal. In support of this, others did not find cocaine-induced sensitization of ACTH responses during or up to 1 week after cocaine administration (Borowsky and Kuhn, 1991; Levy et al., 1992; Torres and Rivier, 1992). Taken together, we postulate that cocaine-induced sensitization of the ACTH response needs at least a week to develop. Nonetheless, it is not irrational to expect that the exact time course is dependent on the exact protocol used for cocaine exposure. The delay in cocaine-induced sensitization resembles that observed after exposure to stressors where long-lasting hyper-responsiveness of the ACTH response (weeks) is preceded by a period of normo-responsiveness of at least several days (Tilders et al., 1993; Van Dijken et al., 1993; Schmidt et al., 1995). In view of these similarities it seems possible that stressor- and cocaine-induced sensitization of ACTH responses are mediated by (yet unidentified) mechanisms.

The delayed sensitization of the ACTH response to cocaine was preceded by sensitization of the corticosterone response which was evident 3 days after cocaine withdrawal. Because the kinetics of the ACTH responses remain unchanged during or shortly after repeated cocaine exposure (Borowsky and Kuhn, 1991; Levy et al., 1992; Torres and Rivier, 1992), this finding indicates that the sensitization of the corticosterone response to cocaine is at least in part due to a non-ACTH-mediated mechanism. Within-groups regression analysis reveals that in rats pretreated with saline 2 weeks earlier the cocaine-induced corticosterone response shows good correlation with the ACTH response ($r = 0.76$, $P = 0.03$), whereas in rats pretreated with cocaine this correlation is absent ($r = 0.19$, $P = 0.67$). This indicates that the adrenal hyper-response to cocaine in these rats remains (at least in part) independent of ACTH during 2 weeks following cocaine withdrawal. Others did not find cocaine-induced sensitization of the corticosterone response for up to one week after cocaine withdrawal (Levy et al., 1992; Torres and Rivier, 1992) which may relate to differences in the experimental conditions as discussed above.

Because saline injection in rats pretreated with cocaine 2 weeks earlier increases plasma ACTH and corticosterone levels, a response that is not found in saline-pretreated rats, we suggest that sensitization of the hypothalamus-pituitary-adrenal axis to cocaine is paralleled by sensitization towards other stimuli.

In conclusion, our data show for the first time that intermittent exposure to cocaine induces a delayed and long-lasting sensitization of the cocaine-induced ACTH secretion from the pituitary gland. In addition, sensitization of cocaine-induced corticosterone secretion shortly after repeated drug exposure is at least in part not ACTH mediated. Because corticosterone has been shown to facilitate self-administration of drugs of abuse in a dose-dependent manner, the present data lend further support for the hypothesis that corticosterone may be involved in the behavioral sensitizing effects of drugs of abuse (Piazza et al., 1991, 1994). Because acute cocaine-induced ACTH release is dependent on CRH release from hypothalamic CRH neurons (Rivier and Vale, 1987; Rivier and Lee, 1994) it is likely that the delayed enhanced ACTH response depends on slowly developing neuroadaptive changes in hypothalamic CRH neurons and/or of other brain centres. In support of the latter possibility, it is worth noting that cocaine-induced changes in prolactin secretion also developed with a delay (present study), suggesting that delayed adaptive changes may occur in a neuronal substrate controlling both the hypothalamic CRH neurons (controlling ACTH) and the tuberoinfundibular dopaminergic neurons (controlling prolactin). These findings may reflect alterations in dopaminergic and/or serotoninergic neurotransmission as proposed by Levy et al. (1994). In this respect it is worth noting that a previous study from our laboratory revealed that intermittent morphine exposure induced a delayed and long-lasting increase in dopaminergic neurotransmission in rat striatum (Tjon et al., 1994). This delayed appearance of central neuroadaptive effects observed long after cessation of drug (or stress) exposure represents a novel aspect of adaptive processes in the brain.

Acknowledgements

We thank Mr G. Wardeh for his expert technical assistance. This work was supported by a grant from

the Dutch Foundation for Scientific Research (Nederlands Wetenschappelijk Onderzoek, NWO grant number: 900-543-101).

References

Borowsky, B. and C.M. Kuhn, 1991, Chronic cocaine administration sensitizes behavioral but not neuroendocrine responses, Brain Res. 543, 301.

Levy, A.D., Q. Li, M.C. Alvarez Sanch, P.A. Rittenhouse, J.E. Kerr and L.D. Van der Kar, 1992, Neuroendocrine responses to cocaine do not exhibit sensitization following repeated cocaine exposure, Life Sci. 51, 887.

Levy, A.D., M.H. Baumann and L.D. Van de Kar, 1994, Monoaminergic regulation of neuroendocrine function and its modification by cocaine, Frontiers Neuroendocrinol. 15, 85.

Piazza, P.V., J.M. Deminière, M. Le Moal and H. Simon, 1990, Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration, Brain Res. 514, 22.

Piazza, P.V., S. Maccari, J.M. Deminière, M. Le Moal, P. Mormède and H. Simon, 1991, Corticosterone levels determine individual vulnerability to amphetamine self-administration, Proc. Natl. Acad. Sci. USA 88, 2088.

Piazza, P.V., M. Marinelli, C. Jodogne, V. Deroche, F. Rouge-Pont, S. Maccari, M. Le Moal and H. Simon, 1994, Inhibition of corticosterone synthesis by metyrapone decreases cocaine-induced locomotion and relapse of cocaine self-administration, Brain Res. 658, 259.

Rivier, C. and S. Lee, 1994, Stimulatory effect of cocaine on ACTH secretion: role of the hypothalamus, Mol. Cell. Neurosci. 5, 189.

Rivier, C. and W.W. Vale, 1987, Cocaine stimulates adrenocorticotropin (ACTH) secretion through a corticotropin-releasing factor (CRF)-mediated mechanism, Brain Res. 422, 403.

Robinson, T.E. and K.C. Berridge, 1993, The neural basis of drug craving: an incentive-sensitization theory of addiction, Brain Res. Rev. 18, 247.

Schmidt, E.D., A.W.J.W. Janszen, F.G. Wouterlood and F.J.H. Tilders, 1995, Interleukin-1 induced long-lasting changes in hypothalamic corticotropin-releasing hormone (CRH)-neurons and hyperresponsiveness of the hypothalamus-pituitary-adrenal-axis, J. Neurosci. (in press).

Tilders, F.J.H., Schmidt, E.D. and D.C.E. DeGoeij, 1993, Phenotypic plasticity of CRF neurons during stress [Review], Ann. NY Acad. Sci. 697, 39.

Tjon, G.H.K., T.J. De Vries, E. Ronken, F. Hogenboom, G. Wardeh, A.H. Mulder and A.N.M. Schoffelmeer, 1994, Repeated and chronic morphine administration causes differential long-lasting changes in dopaminergic neurotransmission in rat striatum without changing its δ- and κ-opoid receptor regulation, Eur. J. Pharmacol. 252, 205.

Torres, G. and C. Rivier, 1992, Differential effects of intermittent or continuous exposure to cocaine on the hypothalamic-pituitary-adrenal axis and c-fos expression, Brain Res. 571, 204.

Van Dijken, H.H., D.C.E. DeGoeij, Sutanto, J. Mos, E.R. De Kloet and F.J.H. Tilders, 1993, Short inescapable stress produces long-lasting changes in the brain-pituitary-adrenal-axis of adult male rats, Neuroendocrinology 58, 57.

Van Oers, J.W.A.M., J.P. Hinson, R. Binnekade and F.J.H. Tilders, 1992, Physiological role of corticotropin-releasing factor in the control of adrenocorticotropin-mediated corticosterone release from the rat adrenal gland, Endocrinology 130, 282.