ELSEVIER

Pharmacology, Biochemistry and Behavior 67 (2000) 25–28

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

Plasma concentrations of beta-endorphin in smokers who consume different numbers of cigarettes per day

J.L. del Arbol[a,*], J. Raya Muñoz[a], L. Ojeda[a], A. López Cascales[a], J. Rico Irles[a], M.T. Miranda[b], M.E. Ruiz Requena[c], J.C. Aguirre[a]

[a]*Departamento de Medicina, Facultad de Medicina, Universidad de Granada, E-18071 Granada, Spain*
[b]*Departamento de Bioestadística, Facultad de Medicina, Universidad de Granada, E-18071 Granada, Spain*
[c]*Departamento de Bioquímica, Facultad de Medicina, Universidad de Granada, E-18071 Granada, Spain*

Received 26 October 1999; received in revised form 15 March 2000; accepted 20 March 2000

Abstract

The harmful effects of smoking on health have been widely documented, although it is as yet unclear whether tobacco dependence is only psychological in nature, or both psychological and physical. We studied plasma concentrations of beta-endorphin, cortisol, and adrenocorticotropic hormone (ACTH) in healthy persons who consumed different numbers of cigarettes per day, and compared the findings with those in a control group of nonsmokers. Beta-endorphin levels were significantly higher than in controls only in persons who smoked fewer than 10 cigarettes per day. Cortisol levels were significantly higher in smokers who consumed more than 20 cigarettes per day. There were no significant differences between any of the groups in plasma ACTH concentrations.

Keywords: ACTH; Beta-endorphin; Cortisol; Smoking; Cigarettes consumed per day

1. Introduction

Nicotine is known to release neuroendocrine substances that may subsequently reinforce smoking behavior by improving mood states. Since the discovery of the endogenous opioid system, many studies have searched for correlations between β-endorphin (β-end) levels and smoking or nicotine dependence [3,12,25]. Most of them demonstrated elevated plasma levels of β-end under a variety of experimental conditions, for example, in women smokers [2], when high doses of nicotine were given [11], after rapid smoking of two high-nicotine cigarettes [13], and in heavy smokers only [8]. Other studies reported that the opiate system might play a minor role in nicotine dependence [16], or found no changes in β-end levels [26]. These discrepancies may be related, in part, with differences in the route of administration of nicotine, dose, experimental animal, or duration of the study. Moreover, most studies did not distinguish between heavy, moderate, and light smokers.

To help find the reasons for the discrepancies between earlier studies, we measured serum concentrations of β-end, cortisol, and adrenocorticotropic hormone (ACTH) in persons who habitually consumed different numbers of cigarettes per day. We assumed that nicotine levels could vary during the day in a given subject, and that they could vary between subjects within the same group, depending on the circumstances under which a given cigarette was smoked. We also assumed that the number of cigarettes smoked per day was not predictive of nicotine levels.

2. Subjects and methods

The study was approved by our center's ethics committee, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The subjects were 85 men (mean age 41 years, range 18–64 years) who were in good general health and were free of known diseases at the time of the study. All men were employees at the same local company in Granada, Spain. We used a questionnaire to verify that lifestyle characteristics such as stress [17], anxiety, type of employment, and alcohol consumption were

* Corresponding author. Carrera del Genil 35, 7, E-18009 Granada, Spain. Tel.: +34-958-229198; fax: +34-958-291834.

0091-3057/00/$ – see front matter
PII: S0091-3057(00)00291-4

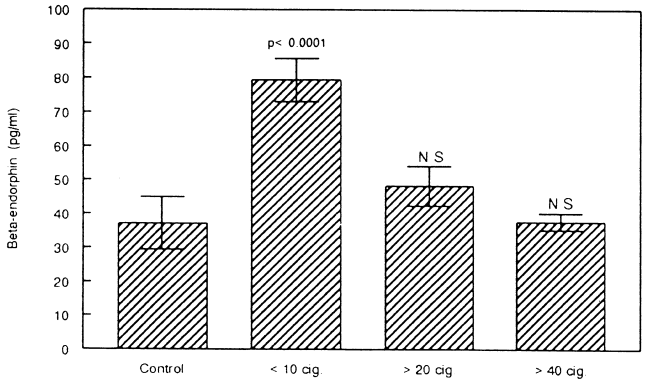

Fig. 1. Plasma concentrations of beta-endorphin in different groups of smokers (according to number of cigarettes smoked per day) and a control group of nonsmokers. NS, not significant.

similar in all subjects, and did not significantly skew the values of the variable we measured.

In the course of a personal interview, the purpose of the study was explained and the subject's motivation to comply with the study procedure was evaluated. All subjects who agreed to take part signed an informed consent form. We obtained a complete personal and family medical history and recorded type of employment, number of cigarettes usually smoked per day, and duration of the smoking habit. Complete laboratory analyses including hemogram, biochemical parameters, urinalysis, β-end, cortisol, and ACTH were done for each participant. A blood sample (8 ml) was obtained between 0800 and 0900 h after an overnight fast (to avoid the influence of the circadian rhythm) [5], during which the subjects also abstained from smoking. The plasma was separated and stored at $-20°C$ until analysis. Plasma nicotine concentration was not measured. The amount of nicotine differed between brands of cigarettes, ranging from 0.9 to 1.1 mg FTC estimated nicotine delivery.

The control group consisted of 22 nonsmokers aged 18 to 53 years (mean age 35 years). Smokers were divided into three groups of 21 subjects each: light smokers, who consumed fewer than 10 cigarettes per day (mean age 41 years, range 18–64 years); moderate smokers, who consumed more than 20 cigarettes per day (mean age 40 years, range 18–62 years); or heavy smokers, who consumed more than 40 cigarettes per day (mean age 40 years, range 18–61 years). Subjects were not offered the option of stating that they consumed 10–20 cigarettes per day, as we assumed that these subjects would report their consumption as "about a pack a day". We excluded from study all subjects who appeared unlikely to cooperate fully with the study, and to avoid the possible influence of drugs on hormonal analyses we excluded any subject who was taking medication (analgesics, anxiolytics, etc.).

Beta-endorphin in plasma was measured by immune radiometric assay (IRMA) with the Nichols Institute diagnostic kit (San Juan de Capistrano, CA); normal values were 29–40 pg/ml. For ACTH, we used the K-PR ELSA-ACTH radioimmunoassay kit from Cis-Industrie, SA (Gif-sur-Yvette, France); normal values were 16–60 pg/ml. Cortisol concentration was measured with the Cort-CTK-2 radioimmunoassay kit from Sorin Biomedica SPA (Salveggia, Italy); normal values were 16 ± 5 μg/dl. The sensitivity of each method was 16 pg/ml, 2.7 pg/ml, and 4.5 μg/dl, respectively, and the specificity of the test used to measure β-end concentration was considered very high (16% cross-reaction with beta-lipoprotein).

Descriptive statistics were used for each of the three substances, and normality of the distribution of the values for each was checked with the Kolmogorov–Smirnov test, which detected no significant variations. The four groups were then compared with analysis of variance (ANOVA). Because the variances of the data for β-end and ACTH were unequal, we subjected these data to logarithmic transformation to ensure homogeneity of the variances. When ANOVA revealed significant differences between groups, we used the Newman–Keuls test for multiple comparisons and the Scheffe test. For all multiple comparisons the global α error was 0.05.

3. Results

Of the three substances we compared in smokers and nonsmokers, the findings for β-end were of greatest interest. Plasma β-end concentration was significantly higher in smokers who consumed fewer than 10 cigarettes per day (79.46 ± 6.31 pg/ml, $p < 0.0001$ in comparison with the value in control subjects: 37.11 ± 4.76 pg/ml); smokers who consumed 20–40 cigarettes per day (48.26 ± 5.80 pg/ml), and smokers who consumed more than 40 cigarettes per day (37.73 ± 2.41 pg/ml). The difference between moderate and heavy smokers was not significant, nor did β-end concentration in either of these groups differ significantly in comparison with the mean concentration in the control group. Fig. 1 illustrates the plasma levels of β-end in each group.

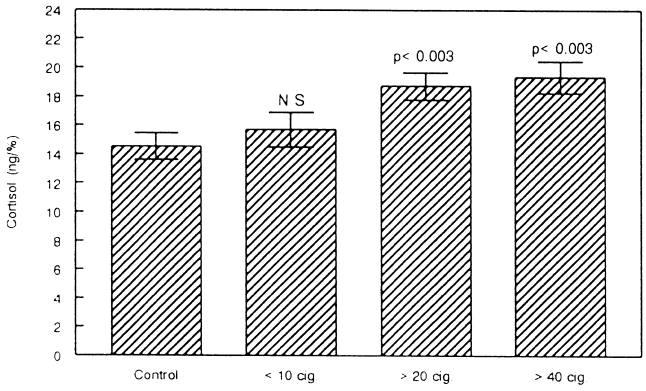

Fig. 2. Plasma concentrations of cortisol in different groups of smokers (according to number of cigarettes smoked per day) and a control group of nonsmokers. NS, not significant.

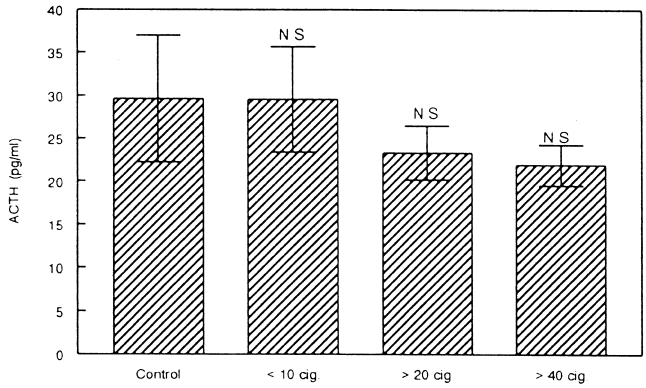

Fig. 3. Plasma concentrations of ACTH in different groups of smokers (according to number of cigarettes smoked per day) and a control group of nonsmokers. NS, not significant.

The results of ANOVA showed significant differences between smokers who consumed more than 20 cigarettes per day and those who consumed more than 40 cigarettes per day in plasma cortisol concentrations. Further analysis with the Newman–Keuls test showed a significant difference between moderate (18.71 ± 0.95 μg/dl) and heavy smokers (19.36 ± 1.09 μg/dl), and between nonsmokers (14.52 ± 0.91 μg/dl) and light smokers (15.69 ± 1.21 μg/dl ($p < 0.003$). As Fig. 2 shows, the number of cigarettes smoked per day correlated directly with plasma cortisol levels.

Although ACTH concentration decreased as the number of cigarettes consumed per day increased, we found no statistically significant differences between any of the groups (Fig. 3). The mean values for each group were: nonsmokers 29.60 ± 7.38, light smokers 29.53 ± 6.17, moderate smokers 23.32 ± 3.14, and heavy smokers 21.91 ± 2.38 pg/ml.

Analysis with the Spearman rank correlation test showed that age was not related with plasma concentration of any of the three substances we investigated.

4. Discussion

Until recently, smoking was considered a social habit, and in some cultures it has been considered beneficial. The harmful effects of smoking on health have been widely documented, although it is as yet unclear whether tobacco dependence is only psychological in nature, or both psychological and physical [26]. The discovery of the endogenous opioid system has led some authors to ask whether this system might be involved in addiction to substances such as tobacco and alcohol [1,4]. This hypothesis is based on the finding that opiate receptors can bind nicotine or its derivatives. Previous studies of the effects of smoking contain contradictory results, which make it difficult to draw firm conclusions.

The results we obtained are difficult to interpret in the light of earlier findings. A plausible explanation, in the light of the clear demonstration by Seyler et al. [25] of a dose–response relationship between nicotine levels and β-end in heavy smokers, and the subsequent replication by Gilbert et al. [8], is that increases in β-end levels are sustained longer (overnight) in lighter smokers than in heavier smokers. Only the group of light smokers (fewer than 10 cigarettes per day) showed a significantly higher level of β-end in comparison to nonsmokers, moderate smokers, and heavy smokers. One earlier study [13] measured β-end after subjects had smoked two, four, or five cigarettes at 30-min intervals. They found elevations in β-end immediately after the second cigarette had been consumed, but not after the fourth or fifth cigarette. However, their sample size was relatively small (16 nonsmokers and 16 smokers of at least 15 cigarettes per day), and their experimental design is not comparable to ours.

Gilbert et al. [8] found that an increase in β-end was related with increased circulating levels of nicotine. However, their experimental design also differed from ours in that they did not measure nonsmoker control concentrations of β-end. We can, nevertheless, surmise, on the basis of the findings in our subjects, that basal circulating concentrations of this hormone in their subjects who smoked more than 15 cigarettes per day were within the normal range.

Our findings may be further evidence of a relationship between elevated levels of β-end and possible pleasurable sensations not measured in this study. The members of our group of light smokers, in whom basal β-end levels were higher than in other groups, may not need to smoke more to obtain the pleasing effects of increased β-end levels. In contrast, moderate and heavy smokers fail to attain this pleasurable basal level of hormone, and may need to smoke more so that the increased nicotine level brings about the transient increase in circulating β-end seen soon after a cigarette is smoked [7,13]. This fact suggests that just as there are individual differences in the response to and dependence on nicotine [18], there may be individual differences in the response of β-end to nicotine, its derivatives, or other components of cigarette tobacco, as occurs with alcohol.

The differences in behavior between light, moderate, and heavy smokers may be related with the effect of nicotine on nicotinic cholinergic receptors. At low doses, nicotine stimulates acetylcholine receptors, whereas high doses of nicotine selectively blocks them [23], an effect that leads to an increase in dopamine production. Because dopamine is a β-end antagonist [14], increased dopamine production may account for the normal levels of β-end we found in persons who smoked more than 20 cigarettes per day. However, this hypothesis will require further study and confirmation, because the number of cigarettes smoked per day does not reliably predict nicotine levels [10]. Peripheral blood concentrations of β-end do not always correlate highly with central concentrations [6,21], and use of the opioid antagonist naloxone does not appear to modify smoking [15], although some authors have reported positive results [9,19].

Plasma concentrations of cortisol also differed between the groups of smokers we studied; the differences were greater between moderate and heavy smokers than between control subjects and light smokers. These results were confirmed by the stricter criteria of the Scheffe test. Cortisol levels were significantly elevated in persons who smoked more than 20 or more than 40 cigarettes per day in comparison with nonsmokers and persons who smoked fewer than 10 cigarettes per day. The greater the number of cigarettes consumed, the greater the increase in plasma cortisol concentration. These results contrast with the findings for β-end, a dissociation also reported by other authors [8,13,20,24]. Several different explanations for this discrepancy have been suggested, including a possible direct action of nicotine on the adrenal cortex [22].

Our results for ACTH show that although plasma levels of this hormone decreased more in moderate and heavy smokers, the decrease in comparison with control subjects was not significant. This finding may be related with a cortisol feedback mechanism.

We conclude that our findings are evidence in support of the hypothesis that nicotine addiction has both biological and psychological components, and that some of these reinforcement effects may be mediated by the opioid system. However, the lack of data on plasma nicotine levels limits the inferences that can be drawn, as self-reported number of cigarettes smoked per day may not fully reveal actual nicotine exposure.

Acknowledgments

We thank the staff of the biochemistry laboratory at the Hospital Universitario San Cecilio de Granada for their help, and Karen Shashok for translating parts of the original manuscript into English.

References

[1] Aguirre JC, del Arbol JL, Raya J, Rico J, Ruiz Requena ME. Effects of acute alcohol intoxication on the opioid system in humans. Alcohol 1995;12:559–62.

[2] Backon J. Negative correlation of cigarette smoking and dysmenorrhea: reduced prostaglandin synthesis due to beta-endorphin, nicotine or acrolein antagonism. Med Hypotheses 1989;28:213–4.

[3] Dalayeun JF, Nores JM, Bergal S. Physiology of beta-endorphin. A close-up view and a review of the literature. Biomed Pharmacother 1993;47:311–20.

[4] Del Arbol JL, Aguirre JC, Raya J, Rico J, Ruiz Requena MA, Miranada MT. Plasma concentration of beta-endorphin and cortisol in drinking and abstinent chronic alcoholics. Alcohol 1995;12: 525–9.

[5] Fachinetti F, Grasso A, Petragua F, Parrini D, Volpe A, Genezzani AR. Impaired circadian rhythmicity of beta-lipotropin, beta-endorphin and ACTH in heroin addicts. Acta Endocrinol 1984;105:149–55.

[6] Fink AD. Opiate receptors and endogenous opioid peptides. Curr Opin Anaesthesiol 1989;2:428–33.

[7] Gilbert DG, Meliska CJ, Plath LC. Noise stress does not modulate effects of smoking/nicotine on beta-endorphin, cortisol, ACTH, glucose and mood. Psychopharmacology 1997;130:197–202.

[8] Gilbert DG, Meliska CJ, Williams CL, Jensen RA. Subjective correlates of cigarette-smoking-induced elevations of peripheral beta-endorphin and cortisol. Psychopharmacology 1992;106:275–81.

[9] Gorelick DA, Rose J, Jarvik JE. Effects of naloxone on cigarette smoking. J Subst Abuse 1989;1:153–9.

[10] Herning RI, Jones RT, Benowitz NL, Mines AH. How a cigarette is smoked determines blood nicotine levels. Clin Pharmacol Ther 1983;33:84–90.

[11] Jensen RA, Gilbert DG, Meliska CJ, Landrum TA, Szary AB. Characterization of a dose–response curve for nicotine-induced conditioned taste aversion in rats: relationship to elevation of plasma beta-endorphin concentration. Behav Neural Biol 1990;53:428–40.

[12] Matta SG, Beyer HS, McAllen KM, Sharp BM. Nicotine elevates rat plasma ACTH by a central mechanism. J Pharmacol Exp Ther 1987;243:217–26.

[13] Meliska CJ, Gilbert DG. Hormonal and subjective effects of smoking the first five cigarettes of the day: a comparison in males and females. Pharmacol Biochem Behav 1991;40:229–35.

[14] Myer RD. Editorial. Alcohol 1996;13:1–2.

[15] Nemeth-Coslett R, Griffiths RR. Naloxone does not affect cigarette smoking. Psychopharmacology 1986;89:261–4.

[16] Olson GA, Olson RD, Kastin AA. Endogenous opiates: 1995. Peptides 1996;17:1421–66.

[17] Owen PC, Smith R. Opioid peptides in blood and cerebrospinal fluid during acute stress. Clin Endocrinol Metab 1987;1:415–35.

[18] Pianezza ML, Sellers EM, Tyndale RF. Nicotine metabolism defect reduces smoking. Nature 1998;393:750.

[19] Pomerleau OF. Endogenous opioids and smoking: a review of progress and problems. Psychoneuroendocrinology 1998;23(2): 115–30.

[20] Pomerleau OF, Fertig JB, Seyler LE, Jaffe J. Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology 1983;81:61–7.

[21] Post RM, Gold P, Rubinow DR, Ballenger JC, Bunney WE, Goodwin FK. An approach to central nervous system peptide function. Life Sci 1982;31:1–15.

[22] Robin RP, Warner W. Nicotine-induced stimulation of steroidogenesis on adrenocortical cells of the cat. Br J Pharmacol 1975;53:357–62.

[23] Sastry BU. Neuropharmacology of nicotine: effects on the autoregulation of acetylcholine release by substance P, and methionine enkephalin in rodent cerebral slices and toxicological implications. Clin Exp Pharmacol Physiol 1995;22:288–90.

[24] Seyler LE, Fertig JB, Pomerleau OF, Hunt D, Parker K. The effects of smoking on ACTH and cortisol secretion. Life Sci 1984;34:57–65.

[25] Seyler LE, Pomerleau OF, Fertig JB, Hunt D, Parker K. Pituitary hormone response to cigarette smoking. Pharmacol Biochem Behav 1986;24:159–62.

[26] Wewers ME, Tejwani GA, Anderson J. Plasma nicotine, plasma beta-endorphin and mood states during periods of chronic smoking, abstinence and nicotine replacement. Psychopharmacology 1994; 116:98–102.