Psychopharmacology (1989) 98:539–543

Psychopharmacology

Neuroendocrine responses to nicotine and stress: enhancement of peripheral stress responses by the administration of nicotine

David E. Morse*
Department of Medical Psychology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

Abstract. Habitual smokers frequently report that when they are stressed smoking helps them to relax. One potential explanation for the reported stress ameliorating effect of smoking is that cigarette consumption (nicotine self-administration) may decrease the sympathetic autonomic nervous system activity which is associated with the stress response. In the present study, rabbits prepared with chronic vascular cannulae were used to study the effects of nicotine administation on plasma corticosterone, catecholamine (epinephrine, norepinephrine and dopamine) and glucose responses to physical restraint stress. Nicotine (0.025, 0.05 or 0.10 mg nicotine base/kg body weight) was administered for 10 days prior to the "stress test" to allow for the development of habituation/tolerance to its acute toxic effects. Independent administration of nicotine, or the application of the physical restraint stressor, resulted in increases in the plasma concentrations of corticosterone, epinephrine, norepinephrine, and glucose. Nicotine administration during restraint stress enhanced the increase in plasma corticosterone and epinephrine, as compared to the responses induced by either factor alone. The results suggest that the stress ameliorating effect of continued cigarette smoking, as reported by habitual smokers, is not due to a reduction in the activity of the peripheral sympathetic autonomic nervous system.

Key words: Nicotine – Stress response – Cigarette smoking

Habitual cigarette smokers frequently report that when they are under stress continued smoking helps them to relax (McArthur et al. 1958; Coan 1973). Further, stress and anxiety appear to be potent environmental cues for cigarette smoking among long-term tobacco users (Emery et al. 1968; Ikard et al. 1969; Frith 1971). The results of several studies suggest that smokers increase the intensity of smoking (i.e., increase the number of cigarettes consumed, or the number and depth of puffs taken) when confronted with a variety of environmental stressors (i.e., loud noise, electric shock, or fear of public speaking: Schachter et al. 1977; Golding and Mangan 1982; Rose et al. 1983).

In addition, it has been reported that chronic smokers who were allowed to smoke were willing to endure more intense electrical shocks or were less annoyed by loud noises than were smokers who were not allowed to smoke or were allowed to smoke only low nicotine content cigarettes during testing (Schachter 1978; Silverstein 1982). In these studies it was assumed that the more anxious the subject the less shock or noise they would be willing to endure. Thus, if smoking/nicotine has an anti-anxiety effect, the smoker allowed to smoke should tolerate more shock or louder noise than the smoker not allowed to smoke. At least superficially, the experimental evidence appears to corroborate the reports of habitual cigarette smokers; i.e., continued smoking during periods of stress "appears" to decrease the aversive impact of the stressor.

Nicotine, the primary phamacological and addictive agent in tobacco, is a potent sympathomimetic stimulant (Taylor 1980; USDHHS 1988). Administration of nicotine in doses similar to those obtained in smoking produced increases in heart rate, systolic and diastolic blood pressure, and activation of the EEG in humans and many animal species (Larson et al. 1961; Larson and Silvette 1968, 1975; USDHHS 1988). Thus, the reported stress ameliorating effect of continued smoking (nicotine administration) in the habitual tobacco user is contrary to expectation considering the pharmacology of nicotine. In fact, this conundrum has been given the name "Nesbitt's Paradox" (Schachter 1973).

Several potential explanations exist for the reported stress ameliorating effect of smoking (nicotine administration) during stress: a) smoking (nicotine) may decrease the intensity of sympathetic autonomic arousal which is associated with the stress response; b) failure of the habitual smoker to continue smoking while stressed may result in physiological and/or psychological withdrawal symptoms which may add to the intensity of the stress response; c) stress responses may alter the relative bioavailability or effective dose of nicotine, thereby inducing a condition of "relative" nicotine withdrawal or craving (cf., Grunberg et al. 1983); or d) continued smoking may serve as a "psychological tool" (Ashton and Stepney 1982), providing the smoker with an alternate focus of attention and/or a causal object for the mis-attribution of the stress mediated arousal.

The present study made use of an animal model of tobacco smoking to examine the interaction of an environmental stressor (physical restraint) and the administration of nicotine on the response of several stress sensitive neuroendocrine systems (plasma catecholamines, corticosterone and glucose). Changes in glucocorticoids and catecholamines are recognized indices of the stress response in humans and many animal species (Baum et al. 1983). Circulat-

* *Current address:* Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145, USA

ing glucose levels, while not necessarily a direct measure of stress, are generally recognized to be highly responsive to changes in adrenomedulary function and the sympathetic autonomic nervous system. Nicotine was administered by constant rate influsion on a chronic intermittent basis (4 times per day). Assuming a half-life of approximately 2 h, this administration paradigm allowed for the examination of both acute infusion-induced responses and cumulative dosing effects, as are evident in the human smoker.

Based on the reports of cigarette smokers, it was hypothesized that nicotine administration (cigarette smoking) reduces the physiological arousal associated with the stress response or it relieves the physiological withdrawal symptoms associated with nicotine dependence. Either of these effects would be evident as a reduction in plasma corticosterone, catecholamine and glucose responses when nicotine was administered during stress as compared to the responses induced by application of the stressor alone.

Methods

Subjects. Twelve male New Zealand White rabbits (*Oryctolagus cuniculus*; Hazelton-Dutchland Laboratory Animals, Inc.) were used. Pre-experimental body weight for each animal was 3.0–3.5 kg. Three animals were randomly assigned to each of the four experimental conditions (i.e., three nicotine dosages and saline control), with the stipulation that the group mean body weights be statistically equivalent.

Animals were individually housed (stainless steel cages, $61 \times 61 \times 37$ cm height), and maintained on a 12 h light/dark cycle beginning at 0600 hours (EST), with a temperature of $20 \pm 1°$ C and relative humidity of 50%. Rabbit diet (Charles River) and water were availble ad lib., except that food was withdrawn 8 h preceding drug sessions during which blood samples were to be taken (i.e., drug administration days 7, 11 and 13).

Pre-surgical procedures. A 1-week gentling period was conducted prior to the experiment. Following this, measurements were made of the body weight of each animal on 4 consecutive days. Weight was computed as the average of ten individual weighings. An average of the daily weights was then computed for individual animals and, the value used subsequently to compute drug dosages.

Antibiotic therapy (30000 units bicillin/kg (IM) and 4 mg gentamicin sulfate/kg (IM)) was instituted 3 days prior to surgery. Gentamicin was administered on a daily basis for the duration of the experiment. Bicillin was administered 3 days before and immediately prior to surgery only. Injections were given at 1800 hours each day. Antibiotics were administered as a precaution to reduce the likelihood of infection in the catheterized animals.

Surgical procedure. Animals were anesthetized with ketamine HCl (45 mg/kg; Parke, Davis and Co.) and xylazine (2.5 mg/kg; Cutter Laboratories). Additional doses of ketamine (5–10 mg/kg) were administered as required during surgery to maintain anesthesia.

Cannulations (Intramedic PE-60 polyethylene tubing) of the left jugular vein and carotid artery were performed under aseptic conditions. A catheter was inserted in each vessel through a non-transecting cut, with the tip of the catheter advanced approximately 4–6 cm and directed toward the heart. The catheters were extended to a point overlying the scapular region, where they were exteriorized. Following surgery, each animal was fitted with an equipment vest and stainless steel catheter harness (Model 410-M, Spalding Medical Products) designed to protect the catheters and incision, yet provide normal freedom of movement. The arterial and venous catheters were connected to a three-channel fluid swivel (Model 310) attached to the roof of the animals' home cage. The fluid swivel was in turn connected to intravenous fluid reservoirs located outside the cage.

Drug administration. Nicotine and physiological saline solution containing heparin (Lypho-Med, Inc.) were administered via the chronic indwelling intravenous and intra-arterial catheters, respectively. Drug dose and fluid infusion volume were controlled by two peristaltic pumps (Manostat Corp). Operation of the arterial catheter pump system was continuous, while intermittent operation of the intravenous catheter pump was regulated by a 24-h multiple programmable timer (Lindburg Enterprises).

Nicotine hydrogen tartrate (FW = 498.44 with $2\ H_2O$; BDH Chemicals Ltd.) was dissolved in sterile physiological saline solution to make the following drug dosages: 0.025 mg, 0.050 mg and 0.100 mg/kg body weight (computed as nicotine base). Solutions were adjusted to pH 7.4 by the addition of NaOH and then filtered (Millipore, 0.22 meter pore size). Selection of nicotine doses was based on a review of the available literature and pre-testing of the experimental procedures.

The nicotine or saline solutions were administered by constant rate intravenous infusion during each 20-min drug period (0.075 ml/min, total volume 1.5 ml). Drug administration occurred at 2-h intervals between 0900 and 1600 hours (i.e., 4 times per day; sessions beginning at 0900, 1100, 1300 and 1500 hours). Nicotine was administered on a daily basis for 13 consecutive days. This mode of administration allowed for investigation of the acute biochemical effects of nicotine in animals receiving chronic intermittent exposure.

Nicotine and saline/heparin solutions were administered through the venous catheters. Arterial catheters were used exclusively for the acquisition of blood samples. Between 1600 and 0900 hours the catheters were maintained on a slow infusion of physiological saline solution containing heparin to prevent clotting (flow rate = 0.50 ml/h, heparin = 25 units/ml). A valve system located in the infusion tubing allowed for withdrawal of blood samples and selection of drug infusion solution.

Blood samples. Four samples were obtained during individual stress/nicotine drug sessions. Samples were taken at time zero (immediately prior to nicotine administration) and at 15, 45, and 90 min following the initiation of the second of the daily drug infusions. Samples were approximately 3 ml whole blood.

Samples were collected in chilled syringes containing 50 units heparin, immediately transferred to 10 ml polypropylene test tubes and maitained at 4° C (one ice). Within 30 min of collection, all samples were centrifuged at 1500 *g* for 10 min. at 0° C. Aliquots of plasma were stored at −70° C until assayed.

Blood (plasma) samples were taken on days 7, 11 and 13 of nicotine administration.

Stress manipulation. The stressor consisted of total body immobilization (Rabbit Restrainer, Plas-Labs, Inc.) for a period of 2 h, beginning immediately prior to the second of the daily drug infusions. Using a counterbalanced design (with testing being conducted every other day), each animal was stressed with and without the simultaneous administration of nicotine (i.e., if an animal received nicotine during the stress period on day 11, then this animal would be stressed without the simultaneous administration of nicotine on day 13). Data from day 7 (when nicotine was administered without concurrent manipulation of stress levels) were used for comparison with the stress and stress/nicotine induced responses.

Assays. Catecholamines (epinephrine, norepinephrine and dopamine) were measured by a catechol-o-methyl-transferase radioenzymatic procedure adapted from Durrett and Ziegler (1980). Corticosterone was measured by a competitive-binding radioimmunoassay (H-3 RIA, Radioassay Systems Laboratories). Glucose was measured by an enzymatic glucose-oxidase reaction using a Beckman II Glucose Analyzer.

Data analysis. Raw data were first subjected to an ANCOVA procedure, using each animals' pre-session baseline as a covariate. In addition, all measures were converted to change scores (i.e., the pre-session baseline value for each animal was subtracted from all subsequent samples), which were then subjected to repeated measures ANOVA [significant ($P<0.05$) main effects were found for the drug and stress manipulations; however, in no case were there significant interaction effects ($P>0.15$ for all interaction effects)]. Drug, stress, and stress/drug-induced responses were found not to be correlated with each other, nor with baseline values.

Based on the nonsignificant correlation between baseline and treatment induced responses the samples were considered independent and peak treatment responses (maximum deflection from baseline) were analyzed by ANOVA. Maximum stress- and nicotine-induced changes in plasma norepinephrine and epinephrine were observed in the 15-min sample (15 min following application of the restraint stressor and initiation of nicotine infusion). Peak changes in circulating corticosterone levels were measured in the 45-min sample, while maximal changes in plasma glucose were split between the 45- and 90-min post-session samples. The results of the ANOVA procedures are presented here, with Student-Newman-Keuls post-hoc tests (the experiment-wide error rate was defined as 0.05).

Results

The analyses discussed here were performed using the combined data from all levels of nicotine treatment. Separate analyses were performed in which the data for individual nicotine dose conditions were examined. The results obtained from all of the analyses showed the same pattern of treatment effects. For brevity and clarity of presentation, only the results of the combined nicotine dose data are discussed here. However, treatment group means broken down by the individual nicotine dose conditions are presented at the right of the figure for reference.

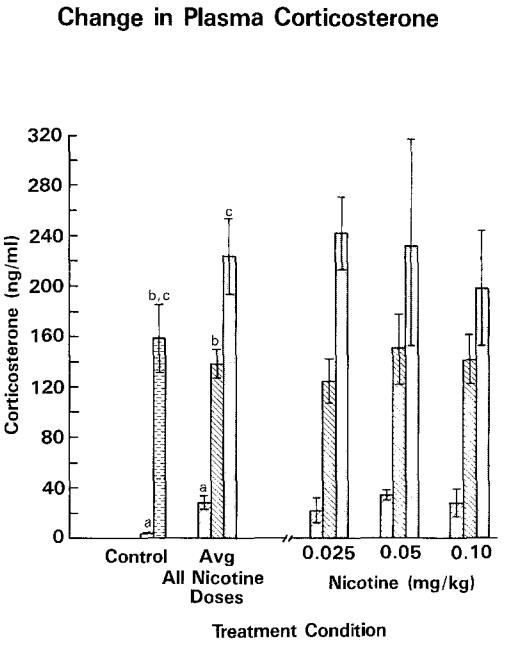

Fig. 1. The administration of nicotine during the period of physical restraint stress increased the plasma concentrations of corticosterone to an extent which was significantly greater than the responses produced by restraint or nicotine alone. The additive and/or synergistic effects of stress and nicotine suggest independent mechanisms of action in the induction of corticosterone responses. No difference in the magnitude of the stress response for the control and nicotine-experienced animals (stress without nicotine) was evident, suggesting that a history of nicotine exposure had not altered the animals' responsiveness to environmental stressors. * Groups with the same letter are not significantly different. □ Control w/o stress; ▤ Control w/stress; ▨ Nicotine; ▧ Stress w/o nicotine; ▩ Stress w/nicotine

Corticosterone

Figure 1 shows the mean change in plasma concentrations of corticosterone for each of the treatment conditions. Although nonsignificant ($P>0.05$), the administration of nicotine resulted in increases in plasma corticosterone concentrations versus the effects induced by the saline control. Physical restraint stress produced significant ($P<0.05$) increases in corticosterone versus the control no-stress condition, the response being similar for the nicotine experienced (Stress w/o Nicotine) and saline control (Control w/Stress) animals. For the nicotine-experienced animals the administration of nicotine during stress (Stress w/Nicotine) resulted in significantly greater increases in the plasma levels of corticosterone than were induced in the same animals by the stressor or nicotine alone. In the overall analysis a significant main effect was found for the treatment condition [$F(4,28)=19.51$, $P<0.0001$].

Catecholamines

Figures 2 and 3 present the mean change in plasma concentrations of epinephrine and norepinephrine for each of the drug treatment conditions. Administration of nicotine during stress resulted in significant increases in epinephrine versus the control and nicotine treatment group [$F(4,28)=3.13$, $p<0.03$] (see Fig. 2). While the administration of nicotine or the application of the stressor alone produced increases in circulating epinephrine levels versus control, these

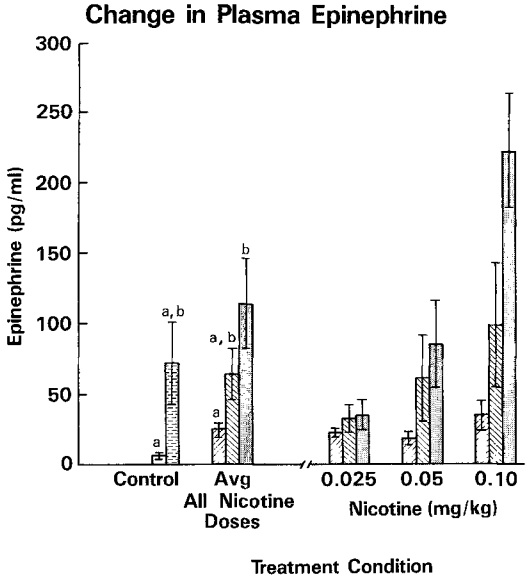

Fig. 2. Nicotine administration during restraint stress significantly increased epinephrine levels versus the control and nicotine treatment effects. The data suggest that the enhancement of the stress response was positively related to the dose of nicotine administered, although this effect was not significant ($P=0.15$). There was no difference in the stress-induced responses of the control animals (control with stress) and animal with a history of nicotine exposure (stress without nicotine). As with the corticosterone responses, this suggests that prior nicotine exposure had not permanently changed the animals' ability to respond to noxious stimulation. * Groups with the same letter are not significantly different. For symbols see figure legend of Fig. 1

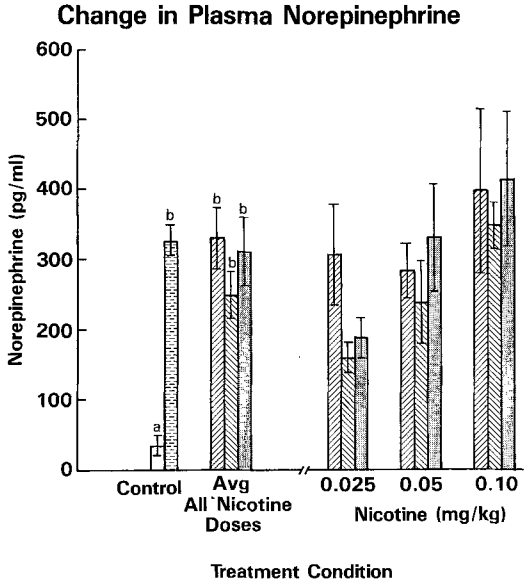

Fig. 3. Restraint stress, nicotine and the combination treatment, all significantly increased plasma norepinephrine levels versus the control (saline administration) conditions. Unlike changes in plasma corticosterone and epinephrine, norepinephrine responses were not significantly enhanced by the combined stress/nicotine treatment. However, it is important to note that norepinephrine responses were not decreased by the administration of nicotine during stress (as compared with the stress induced responses). These data may suggest a common mechanism of action underlying the nicotine- and stress-induced norepinephrine response. * Groups with the same letter are not significantly different. For symbols see figure legend of Fig. 1

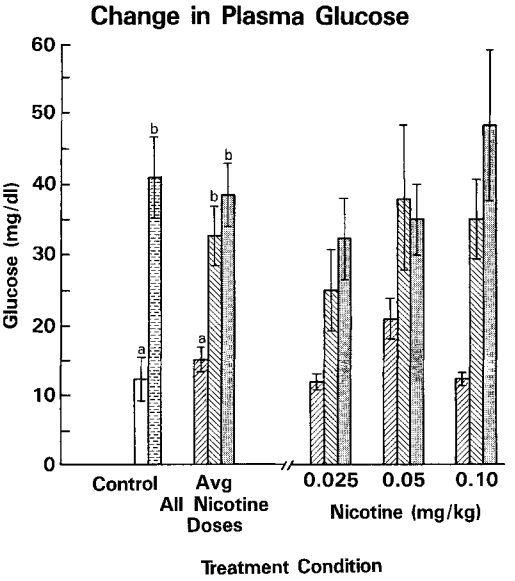

Fig. 4. Plasma glucose levels were significantly increased by restraint stress and the combined stress/nicotine treatment. Among the nicotine-experienced animals, the responses to stress and stress with nicotine were not significantly different. Stress-induced increases in plasma glucose levels were similar for the control and nicotine-experience animals. As described previously, these data suggest that a history of nicotine exposure does not result in permanent changes in the organisms, physiological responsiveness to environmental stimuli. * Groups with the same letter are not significantly different. For symbols see figure legend of Fig. 1

comparisons were not significant. Significant treatment related effects were found in the analysis of norepinephrine responses [$F(4,28)=3.89$, $P<0.01$] (see Fig. 3). Nicotine administration, physical restraint stress and the combination treatment all resulted in significant increases in plasma norepinephrine levels versus control. Comparisons between the norepinephrine responses induced by nicotine, stress and stress with nicotine were all nonsignificant. No significant findings were observed in the analysis of plasma dopamine responses [$F(4,28)=2.10$, ns].

Glucose

Figure 4 presents the mean change in plasma concentrations of glucose for each of the drug treatment conditions. Stress, and the combined stress/nicotine treatment, resulted in significant increases in circulating glucose versus the control and nicotine treatment conditions. Examination of the stress w/o nicotine and stress w/nicotine treatment effects found no significant differences. However, while not statistically significant, in the 0.10 mg/kg condition, the combination treatment did induce larger increases in circulating glucose than those produced by the stressor alone. Nicotine administration did not significantly effect circulating glucose in any of the doses tested. The results of the ANOVA showed significant treatment effects in glucose responses [$F(4,28)=8.44$, $P<0.0001$].

Discussion

Habitual cigarette smokers frequently report that smoking (nicotine administration) during periods of stress helps them to relax. Based on these reports, it was hypothesized that for habituated organisms the administration of nico-

tine (smoking) during periods of strain may reduce the physiological arousal which is associated with the stress response. Within the context of the present study there are several key treatment group comparisons which are central to answering this question. First, did stress significantly increase the circulating levels of corticosterone, the catecholamines and glucose in control (nicotine naive) and nicotine-experienced animals? Secondly, were there any differences in the stress-induced responses displayed by the control and nicotine-experienced animals? And lastly, among the nicotine-experienced animals, if nicotine was administered during stress were the resultant physiological effects greater or lesser in magnitude to those responses induced by the stressor alone?

Physical restraint stress induced significant increases in the plasma concentrations of corticosterone, epinephrine, norepinephrine, and glucose, for both the control and nicotine-experienced animals. Among the nicotine-treated animals, actual nicotine administration resulted in a significant increase only in the plasma concentration of norepinephrine and nonsignificant increases in corticosterone, epinephrine, and glucose levels. Corticosterone, epinephrine, and glucose responses to restraint stress were larger than those produced by nicotine. The neuroendocrine responses induced by restraint stress alone (i.e., without the simultaneous administration of nicotine) were similar for the saline control and nicotine-experienced animals (i.e., those animals which had received 40 infusions of nicotine during the preceding 10 days). Among the nicotine experienced animals, the administration of nicotine during stress resulted in significantly larger increases in the plasma concentrations of epinephrine and corticosterone than the effects produced by the stressor alone.

The results of the present study coincide with the findings of another recent investigation (MacDougall et al. 1983). In that study, habitual cigarette smokers were asked to play a demanding video game, or smoke a high nicotine content cigarette, or do both. Smoking and stress individually resulted in increases in heart rate and systolic and diastolic blood pressure. Furthermore, it was found that if subjects smoked while engaged in the stressful video game, then the measured increases in heart rate and blood pressure were approximately twice the magnitude of the effects induced by either factor alone.

The experimental results suggest that for the habitual smoker, the reported stress ameliorating effect of continued cigarette consumption during periods of stress is *not* due to a reduction in the stress induced activation of the peripheral sympathetic autonomic nervous system. Further, the nicotine-experienced animals displayed; a) stress induced responses similar to those of the control animals when nicotine was not administered, and b) enhance biochemical indices of stress while receiving nicotine concomitant with stress, as compared to their own responses to the stressor alone. This suggests that withdrawal symptoms did not significantly increase the intensity of the stress-associated arousal in nicotine-experienced animals. In addition, these data suggest that nicotine experience and/or tolerance does not permanently change the responsiveness of the autonomic nervous system to environmental stressors.

The results of the present experiment clearly suggest that for the habitual cigarette smoker, any stress amelioration produced by continued smoking during periods of duress is not mediated by a reduction of peripheral physiological activation. Further research needs to examine whether changes in central nervous system activity, the endogenous opioids, or cognitive processes (such as the attribution of arousal) may mediate the purported stress reduction effect of habitual smoking. Thus, Nesbitt' Paradox remains unanswered; how does the administration of nicotine, a powerful peripheral stimulant, apparently promote stress reduction and relaxation?

Acknowledgements. This work was supported by USUHS Protocols CO7223 and TO7229. The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the view of the Department of Defense, or the USUHS.

References

Ashton H, Stepney R (1982) Smoking: psychology and pharmacology. Tavistock, London

Baum A, Grunberg NE, Singer JE (1982) The use of psychological and neuroendocrinological measurements in the study of stress. Health Psychol 1:217–236

Coan RW (1973) Personality variables associated with cigarette smoking. J Personal Soc Psychol 26:86–104

Durrett LR, Ziegler MG (1980) A sensitive radioenzymatic assay for catechol drugs. J Neurosci Res 5:587–598

Emery FE, Hilgendorf EL, Irvin BL (1968) The psychological dynamics of smoking. Tobacco Research Council Report 10, London

Frith CD (1971) Smoking behavior and its relation to the smoker's immediate experience. Br J Soc Clin Psychol 10:73–78

Golding J, Mangan GL (1982) Arousing and de-arousing effects of cigarette smoking under conditions of stress and mild sensory isolation. Psychophysiology 19:449–456

Grunberg NE, Morse DE, Barrett JE (1983) Effects of urinary pH on monkeys' behavioral responses to nicotine. Pharmacol Biochem Behav 19:553–557

Ikard FF, Green DE, Horn D (1969) A scale to differentiate between types of smoking as related to the management of affect. Int J Addict 4:649–659

Larson PS, Silvette H (1968) Tobacco: experimental and clinical studies (Supplement I). Williams & Wilkins, Baltimore

Larson PS, Silvette H (1975) Tobacco: experimental and clincal studies (Supplement III). Williams & Wilkins, Baltimore

Larson PS, Haag HB, Silvette H (1961) Tobacco: experimental and clinical studies. Williams & Wilkins, Baltimore

MacDougall JM, Dembroski TM, Slaats S, Herd JA, Eliot RS (1983) Selective cardiovascular effects of stress and cigarette smoking. J Human Stress 9:13–21

McArthur C, Waldron E, Dickinson J (1958) The psychology of smoking. J Abnorm Psychol 56:267–275

Rose JE, Ananda S, Jarvik ME (1983) Cigarette smoking during anxiety-provoking and monotonous tasks. Addict Behav 8:353–359

Schachter S (1973) Nesbitt's Paradox. In: Dunn WL Jr (ed) Smoking behavior: motives and incentives. Wiley, New York

Schachter S (1978) Pharmacological and psychological determinants of smoking. Ann Intern Med 88:104–114

Schachter S, Silverstein B, Kozlowski LT, Herman CP, Liebling B (1977) Effects of stress on cigarette smoking and urinary pH. J Exp Psychol [Gen] 106:24–30

Silverstein B (1982) Cigarette smoking, nicotine addiction, and relaxation. J Personal Soc Psychol 42:946–950

Taylor P (1980) Ganglionic stimulating and blocking agents. In: Gilman AG, Goodman LS, Gilman A (eds) The pharmacological basis of therapeutics, 6th edn. Macmillan, New York

USDHHS (1988) The health consequences of smoking: nicotine addiction. A report of the Surgeon General, US Government Printing Office, Washington, DC

Received September 2, 1988 / Final version January 13, 1989