Pharmacology Biochemistry & Behavior, Vol. 12, pp. 107–111. Printed in the U.S.A.

Nicotine and Amphetamine: Differential Tolerance and No Cross-tolerance for Ingestive Effects[1]

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Received 4 May 1979

BAETTIG, K., J. R. MARTIN AND W. CLASSEN. *Nicotine and amphetamine: Differential tolerance and no cross-tolerance for ingestive effects.* PHARMAC. BIOCHEM. BEHAV. 12(1)107–111, 1980.—Rats chronically treated twice daily with nicotine (0.4 mg/kg, SC) or d,l-amphetamine (1.5 mg/kg, SC) exhibited different patterns of anorexia, hypodipsia, and body weight loss. Amphetamine-treated rats developed tolerance to these ingestive effects and to weight loss, whereas nicotine-injected rats did not. There was little, if any, evidence for cross-tolerance when the drugs were switched between the two groups. These results indicate that different mechanisms underlie the ingestive effects of nicotine and amphetamine.

Nicotine Amphetamine Tolerance Cross-tolerance Anorexia Decreased body weight Ingestive effects.

NUMEROUS epidemiological studies have noted that tobacco smokers characteristically maintain lower body weights than nonsmokers [26]. Although those studies that include a broad demographical sample are generally consistent in this respect [4, 7, 8], investigations with more selective samples, such as high income groups [30] or college students [40,43], have sometimes failed to yield such results or have even produced evidence that smokers weigh more than non-smokers. Such findings suggest the effect of smoking on body weight may be influenced by social and environmental factors. More consistently, it has been reported that cessation of smoking is followed by weight gain [8, 24, 18, 47], an increased preference for sweet foods and increased food intake, especially between meals [17,48].

Animal studies differ widely with respect to the route of administration, dosage, age of subjects and feeding schedule [26]. Weight reduction and growth retardation were obtained by many authors with inhalation of tobacco smoke [26]. The nicotine containing phase of tobacco smoke has been reported to be necessary for reducing weight, but the remaining fractions of tobacco smoke also have an effect [35]. Only a few studies investigated the effect of chronic injections of low "smoking doses" [3] on body weight and food intake. Erbacher, Grumbrecht and Loeser [16] reported a retardation of growth rate in rats treated with 0.15 mg/kg nicotine over 40 days. Schechter and Cook [39] found decreased body weight in adult rats treated with 0.4 and 0.8 mg/kg nicotine. As food intake was not changed, the authors suggested that the "weight loss without loss of appetite" was probably due to peripheral metabolic actions rather than to anorectic effects of this substance. However, this conclusion is limited by the fact that food intake was measured weekly and, thus, subtle changes may have escaped observation. Nicotine-induced weight reduction, without concomittant anorexia and the subsequent development of tolerance, would be in contrast to the effects of most drugs used clinically for weight control, which rely on their anorectic action to decrease body weight, but such anorectic effects are susceptible to the development of tolerance [33, 42].

The present study compared the effects of semi-chronic nicotine administration on body weight and ingestive behavior with those produced by amphetamine, which is considered prototypical of many anorectic substances. Subsequent evaluation of cross tolerance was done to permit further assessment of possible differences in the mechanism of action of these two drugs.

METHOD

Animals

A total of 20 male RHA/Verh rats, approximately 13 months of age at the start of drug treatment, were used. The rats were individually housed in Macrolon cages (42×13×15 cm) in animal quarters illuminated with neon lights on a 12:12 hr cycle. The animals had continuous access to tap water, but food (Nafag Lab. Pellets, No. 890) was only available during the 4-hr daily experimental period. The rats weighed approximately 390 g at the start of the experiment.

[1]This research was partially supported by a grant from the Swiss Association of Cigarette manufacturers. Requests for reprints should be sent to: K. Baettig, Institut für Verhaltenswissenschaft, ETH-Zürich, Turnerstrasse 1, 8092 Zürich, Switzerland.

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Drug Treatment

Nicotine hydrogen tartrate and d,l-amphetamine sulfate were used and the dosages were calculated as pure substance. Both drugs were dissolved in saline so as to obtain the desired dosages with injection volumes of 1 ml/kg body weight. The single doses of twice daily injected nicotine (0.4 mg/kg, SC) and d,l-amphetamine (1.5 mg/kg, SC) were selected on the basis of previous experimentation with a wider range of doses [4].

Experimental Procedure

Body weight, food intake, and water intake were measured in the three experimental phases: baseline period (1 week), drug treatment (4 weeks) and drug substitution (2 weeks). Half of the rats received twice daily—before and after the measurements of food intake—subcutaneous injections of 0.15 M NaCl (baseline), 0.4 mg/kg nicotine (treatment), and 1.5 mg/kg d,l-amphetamine (substitution) successively in these phases, whereas the remaining rats received 0.15 M NaCl (baseline), 1.5 mg/kg d,l-amphetamine (treatment), and 0.4 mg/kg nicotine (substitution). Each day body weight was measured at the onset of the dark portion of the day-night cycle. The rats were then injected and returned immediately to their home cages which had been provided with a measured amount of food and water. Cumulative food intake was determined for 1 hr and 4 hr and then the food was removed for the remainder of that day and the rats then received their second injection. Water intake was determined only for the entire 4-hr feeding period. Statistical analysis of data averaged by the week was done with two-tailed *t*-tests.

RESULTS

Pronounced differences between the effects of the two drugs appeared during the initial treatment phase of 4 weeks as evident in Fig. 1 which presents the daily mean food intake, water intake, and body weight of the two groups. Body weight reduction reached the same level with the doses of the two drugs selected for this experiment, however, this reduction was maintained over the 4 weeks of nicotine treatment, whereas the amphetamine-induced weight reduction gradually disappeared over the treatment phase. This reduction, as compared to baseline phase, was significant for weeks 2 through 4 for nicotine (p's<0.005), and for only weeks 1 and 2 for amphetamine (p's<0.01), indicating not only a gradual disappearance, but also a more rapid onset of weight reduction with amphetamine than with nicotine. Anorexia, measured 1-hr post-injection was significant for both drugs and for all 4 weeks (p's<0.005). In terms of magnitude (Fig. 1), this 1-hr anorexia was relatively modest and constant with nicotine. With amphetamine it was very considerable for the first two weeks. Thereafter it declined, but without diminishing to the more modest level of nicotine. A further differentiation was seen with 4-hr food intake. Nicotine produced a mild, but significant (p's<0.005), depression only during the first week, followed by normophagia during the remainder of the treatment phase. Amphetamine produced a severe depression of food intake during the first week but this reversed and the rats exhibited significant (p's<0.005) hyperphagia during the final 3 weeks of treatment, that paralleled the gradual disappearance of the initial weight loss. Water intake over the 4-hr feeding period paralleled the drug induced changes observed with 4-hr food intake. Drug substitution during the last 2 weeks produced moderate evidence for unidirectional cross-tolerance of 1-hr food intake from amphetamine to nicotine, but all other measures, 4-hr food intake, water intake and change of body weight showed no signs of cross-tolerance.

DISCUSSION

Both nicotine and amphetamine produced a 1-hr anorectic effect throughout the 4 weeks of drug treatment, but only amphetamine injected rats gradually increased subsequent feeding (4-hr food intake) over these several weeks to a sufficient extent to eliminate the initial loss of body weight.

The results of a number of investigations suggest that post-injection amphetamine anorexia is due to central actions of this drug [23,42]. Neurophysiological evidence for this hypothesis comes from experiments involving the hypothalamic feeding areas which showed that lesions prevent amphetamine anorexia [9], that systemic injection of amphetamine increased neuronal activity [37] and increased the thresholds for eating elicited by electric stimulation [44], and that anorexia can be produced by local injection of the drug into these hypothalamic regions [10]. Neurochemical research has further shown that the central action of amphetamine may be a consequence of catecholaminergic alterations in the brain [42]. This hypothesis is supported by the demonstration of lowered noradrenaline brain content after amphetamine injection [19] and by elimination of amphetamine anorexia after pretreatment with α-methyl-p-tyrosine, which blocks the synthesis of both noradrenaline and dopamine [5]. Furthermore, amphetamine anorexia was prevented by 6-hydroxydopamine injections proximate to the ventral forebrain bundle which resulted in an almost complete depletion of forebrain noradrenaline [1]. In contrast, the neurophysiology and neuropharmacology of postinjection nicotine anorexia is less well documented, but the limited available evidence suggests a similar mechanism of action. Nicotine transiently increases the threshold for eating elicited by hypothalamic stimulation. This effect can be antagonized by mecamylamine, a nicotinic blocker which readily crosses the blood brain barrier, but not by the peripherally acting nicotine blocker hexamethonium [31]. The dopaminergic blocker haloperidol has been found to be even more effective in preventing nicotine anorexia than mecamylamine [14]. Central noradrenergic [6], dopaminergic [21] and serotonergic [38] effects of nicotine have also been reported, but the possible interaction of such effects with feeding behavior have not been directly investigated. Together, these studies suggest that postinjection nicotine anorexia may be due at least in part to the central actions of the drug, as is the case for amphetamine. In the present experiment, both drugs produced a significant transient postinjection anorexia (1-hr food intake) which lasted throughout the several weeks of drug treatment. This effect was modest with nicotine and was not attenuated by any development of tolerance, but stronger and partly attenuated by tolerance with amphetamine. The fact that a short-term unidirectional cross-tolerance from amphetamine to nicotine was observed for 1-hr anorexia could indicate that different mechanisms underlie the short-term anorectic effects of the two drugs, even if both were mediated by central action. However, it is also possible that this is a consequence of the greater magnitude of amphetamine anorexia in comparison to the nicotine anorexia.

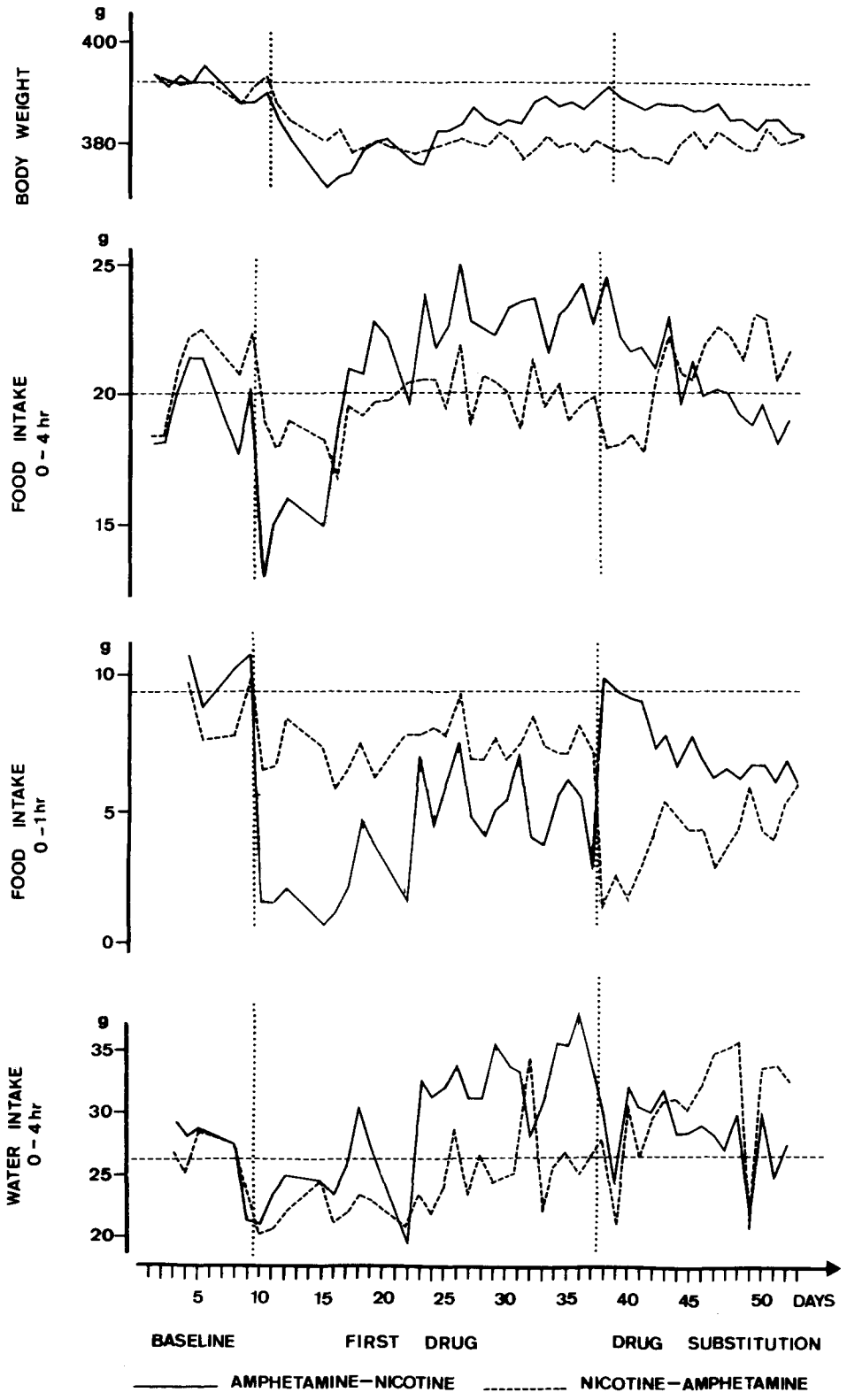

FIG. 1. Daily body weight, food intake and water intake of rats receiving ad lib water and 4-hr daily food access. Half the rats received treatment with amphetamine followed by substitution of nicotine in a cross-tolerance test, and the remaining animals received the drugs in the reverse order. Pretreatment baseline is indicated by the horizontal dashed line.

No cross-tolerance was observed for 4-hr food intake, water intake and development of body weight. Among these measures cumulative food intake over the initial 4-hr postinjection, a period which exceeds the duration of the acute effects of the small doses of drugs used in this experiment, appeared to be the decisive parameter for the differential effect on body weight. Panksepp and Booth [34] discussed the possibility that tolerance may develop when food is continuously available because rats readjust their feeding pattern so as to take advantage of the decay of drug action and to become increasingly food motivated as a consequence of lowered body weight. In terms of this behavioral explanation, the present experiment suggests that for some reason the increase of motivation due to the weight loss induced by amphetamine was greater than that of the weight loss induced by nicotine. With nicotine, 4-hr food intake was gradually readjusted to control levels with maintenance of the weight deficit, whereas with amphetamine, 4-hr food intake was gradually over-compensated with simultaneous disappearance of the initial weight deficit. Although possible neurochemical mechanisms of tolerance have been investigated in recent years, no fully satisfactory explanation has yet emerged. Tolerance of anorexia has been repeatedly demonstrated with chronic amphetamine treatment [28, 34, 46] and alteration of the metabolism of this drug has been excluded as a likely explanation of tolerance [27]. P-hydroxynorephedrine, a metabolite of d-amphetamine which acts as a false transmitter in noradrenergic neurons seems to be an unlikely candidate for explaining tolerance of anorexia [28], since pretreatment with p-hydroxynorephedrine was found to influence the peripheral sympathicotonic effects of amphetamine which are subject to tolerance, but not anorexia, which is also subject to tolerance, or the increased activity level, which is not subject to tolerance. Furthermore, amphetamine induced perseveration was found to be attenuated not only after repeated injections of d-amphetamine but also after treatment with l-amphetamine which is not metabolized to p-hydroxynorephedrine [25]. The fact that different effects of amphetamine are differentially affected by tolerance is further complicated by the demonstration that tolerance for the central catecholaminergic effects of the drug develops more rapidly than for anorexia [29]. Therefore, it has to be assumed that the different mechanisms of tolerance are highly complex and interactive. Interactive neurochemical mechanisms have been proposed by Anisman [2] who discussed the possible role of a compensatory inhibitory and time-dependent cholinergic rebound to catecholaminergic activation in aversively motivated behavior and by Myers [32] who formulated a theory that hypothesizes that food intake is determined by both the central profile of transmitter and neurohumoral factors and the peripheral profile of the ratio of all blood-borne substances. This raises the question of the potential importance of peripheral effects of nicotine and amphetamine in mediating their effects on body weight and ingestive behavior.

Both substances induce sympathicotonic stimulation via adrenal medulla adrenergic activation which results in increased glycogenolysis and lipolysis, but these effects are subject to tolerance. Evidence for altered metabolism was found when either drug was injected, since drug treatment resulted in lower body weight in comparison to isocalorically pair-fed control rats [22, 36, 37]. Thermogenesis has only been reported following amphetamine injection [28] and increases of plasma glucose following only nicotine treatment [14], but both effects were subject to tolerance. More specifically, nicotine might interfere with digestive functions. Small smoking doses increased gastrointestinal secretion in the rat [45] and also increased intestinal mobility in the dog [12]. Other studies suggested that nicotine might interfere with protein metabolism [36]. In pigs, orally administered nicotine produced an increase of lean meat as opposed to fat deposits [15]. On the basis of such results, it is possible that peripheral factors might contribute to maintenance of reduced body weight during a period of nicotine administration. The reports of Schechter and Cook [39] and Passey, Elson and Connors [36] support this view. However, the role of peripheral metabolic actions of smoking in humans has remained controversial [11, 20, 41].

Clinical and epidemiological investigations suggest that the weight reduction generally observed in smokers compared to nonsmokers is maintained throughout the period of smoking but not after cessation [8]. Whether such reductions can be ascribed directly to nicotine or to other factors such as personality or life style of smokers [13] remains to be determined. However, the present results suggest that nicotine may play an important role, acting by a mechanism that is not identical to that of amphetamine.

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