The influence of chronic nicotine treatment on stress-induced gastric ulceration and emptying rate in rats

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Abstract. Ten-day treatment with nicotine (5, 25 or 50 μg/ml drinking water) dose-dependently intensified gastric ulceration induced by cold-restraint, and emptying rate. Stomach contractions produced by graded doses of bethanechol i.v. were elevated further by nicotine treatment. It is suggested that chronic nicotine administration produces hypersensitivity of the gastric muscarinic receptors; stomach hypermotility contributes to the ulcer-worsening action of the alkaloid.
Key words. Nicotine; cold-restraint stress; gastric ulcers and motility.

Nicotine causes many of the pharmacological effects of smoking [1]. Ethanol-induced stomach mucosal damage in rats has been shown to be aggravated by chronic nicotine treatment [2]. A similar effect on gastric ulcers produced by cold-restraint stress in rats has recently been described [3]. The exact mechanism underlying these actions of the alkaloid is still unclear. Since gastric hypermotility is an ulcerogenic factor in rats [4], and gastric emptying rate reflects stomach motility [5], the effects of chronic nicotine treatment on both these parameters have been studied in rats subjected to cold-restraint stress.

Materials and methods

Female Sprague-Dawley rats, weighing 150–170 g, were fed a pellet diet (Ralston Purina Co., USA) and housed in an air-conditioned room (temperature: 22 ± 1 °C; humidity: 65–70%). The animals were randomly divided into groups which drank freely either ordinary tap water (controls) or a solution of nicotine bitartrate (Sigma) 5, 25 or 50 μg/ml tap water for 10 days. Solid food was removed 48 h before experiments, but the rats were allowed free access to a drinking solution of 8% sucrose in 0.2% NaCl w/v (controls) or one containing similar concentrations of the alkaloid. These solutions were removed 1 h before experiments were started. The gastric emptying rate was measured by the method of Brodie and Kundrats [6], 50 amberlite pellets (Rohm & Haas amberlite ion exchange resin IRC-50), 1 mm diameter, in distilled water (5 ml/kg) were given intragastrically via a polythene tube (Portex 6FG). Immediately following this procedure, each rat was stressed by restraint in individual close-fitting cages and exposed to 4 °C; controls were left in their starvation cages at room temperature. All the animals were killed by a sharp blow on the head 1 h later; their stomachs and oesophagi were then removed. The pellets remaining in the gastric lumen were counted and the glandular mucosa examined for lesions [7]. Gastric emptying rate was expressed as the number of pellets expelled during the 1-h observation period.

In another series of experiments, gastric motility in vivo in response to bethanechol stimulations was measured. General anaesthesia, induced with ether (E. Merck), was maintained with chloralose (BDH) 80 mg/kg i.v. and supplemented when needed with further doses of chloralose 10 mg/kg. Following trachea intubation, the duodenum, exposed through an abdominal midline incision, was ligated 2–3 mm distal to the pylorus. An open-ended polythene tube (Portex 5FG) filled with distilled water was then inserted through a cervical-level incision in the oesophagus until its tip entered the stomach; the tube was finally secured by a ligature at its insertion site. Intragastric pressure changes were recorded on a physiograph (MK-IV, Narco Bio-Systems) via a Statham (P23ID) pressure transducer. Following a 10-min stabilisation period, gastric motility was recorded continuously. Graded bolus doses of bethanechol chloride (MSD) were then injected through the cannulated left jugular vein at 15-min intervals. The frequency of stomach contractions, average contraction height and intragastric pressure were measured for 10 min after injection of each dose. The data of all experiments were analysed statistically using the two-tailed unpaired Student t-test.

Results

Nonstressed rats drinking tap water showed a low ulcer index, owing to occasional petechiae in the glandular mucosa; drinking a solution of nicotine 5, 25 or 50 μg/ml tap water for 10 days resulted in ulcer indices which were comparable to those of the tap water-drinking controls (table 1 A). The gastric emptying rate, as reflected by the number of pellets expelled in 1 h, was reduced in nonstressed rats given nicotine 25 or 50 μg/ml ($p < 0.05$ for

Table 1. Effects of 10-day nicotine treatment on gastric ulceration and emptying rate in stressed rats

Treatment	No. of rats	Glandular ulcer index (mm)	No. of pellets expelled
A) No stress (unrestrained at 22 °C for 1 h)			
Tap water	12	0.12 ± 0.06	16.6 ± 2.4
Nicotine 5 μg/ml	12	0.12 ± 0.07	15.8 ± 2.9
Nicotine 25 μg/ml	10	0.20 ± 0.11	10.1 ± 1.8*
Nicotine 50 μg/ml	11	0.16 ± 0.11	10.5 ± 1.5*
B) Stress (restrained at 4 °C for 1 h)			
Tap water	11	2.27 ± 0.32 +	37.3 ± 2.2 +
Nicotine 5 μg/ml	12	2.38 ± 0.58 +	38.2 ± 3.2 +
Nicotine 25 μg/ml	11	3.72 ± 0.58* +	40.7 ± 2.5 +
Nicotine 50 μg/ml	12	5.04 ± 1.07* +	44.3 ± 2.4* +

Values are the means ± SEM. *$p < 0.05$ when compared to its own tap water-drinking control. + $p < 0.001$ when compared to its corresponding nonstressed control in A.

both concentrations). When stressed for 1 h (table 1 B), tap water-drinking controls showed intense haemorrhagic glandular mucosal ulceration. These lesions were concentration-dependently worsened by nicotine; statistical significance was reached with concentrations of 25 or 50 µg/ml ($p < 0.05$ for both). Gastric emptying rate was increased ($p < 0.001$) by stress; nicotine 25 or 50 µg/ml produced a further increase in emptying rate, with the bigger dose reaching statistical significance ($p < 0.05$). As no pellets were found in the oesophagi of any of the rats, those remaining in the stomach after 1 h were, therefore, the balance of the 50 originally delivered intragastrically. Thus, the number of pellets expelled by the stomach was used as an index to evaluate the gastric emptying rate.

Bolus i.v. injections of bethanechol dose-dependently increased the contraction frequency, amplitude and intragastric pressure in rats drinking tap water (table 2 A); a similar pattern of effects was seen after chronic treatment with nicotine 25 µg/ml (table 2 B) or 50 µg/ml (table 2 C). However, only the frequency of stomach contractions produced by the cholinergic agent was found to be exaggerated by nicotine 25 µg/kg (table 2 B; in response to bethanechol 3 µg/kg i.v., $p < 0.05$) or by nicotine 50 µg/ml (table 2 C; in response to bethanechol 3, 10 and 30 µg/kg i.v., $p < 0.05$ for all doses).

Discussion

Although the adverse effects of chronic nicotine treatment on gastric mucosal damage by various ulcerogenic methods have been reported [2, 3, 8], its influence on gastric motility, in relation to lesion formation, is not known. It has been shown that chronic nicotine treatment for 10 days produces ganglion blockade in the stomach wall [9]. Thus, it is possible that such an action could lead to gastric muscarinic receptor supersensitivity, of the type which occurs after denervation; this mechanism would explain past findings [2, 3, 8].

In the current study, stress-induced gastric ulceration was accompanied by more amberlite pellets being expelled by the stomach, indicating an increase in stomach emptying rate. This effect was exaggerated by 10-day nicotine consumption. The observation that gastric emptying was significantly reduced in nonstressed rats drinking nicotine 25 or 50 µg/ml tap water requires further investigation; however, the possibility of ganglion blockade reducing basal stomach wall activity cannot yet be excluded. Gastric muscarinic receptor stimulation by bethanechol produced elevations in the frequency of contraction and amplitude. Ten-day treatment with nicotine 25 or 50 µg/ml drinking water significantly augmented stomach wall contractions, which were directly related to the amount of alkaloid consumed. This action on gastric motility could partly or wholly explain why nicotine worsens stress-induced mucosal ulceration, because an increase in gastric contractions, with subsequent mucosal microcirculatory changes, has been shown to lead to ischaemia and mucosal lesion formation in rats [10–12]. Although further studies are needed, the results reinforce the idea that supersensitivity of the muscarinic receptors could indeed occur after chronic nicotine intake; it is likely that this phenomenon is the consequence of chemical denervation due to ganglionic blockade by the alkaloid.

Table 2. Effects of 10-day nicotine treatment on gastric motility induced by bethanechol in rats

Groups	Bethanechol (µg/kg i.v.) 0	3	10	30	100
A) Rats drinking tap water (n = 12)					
Frequency (contractions/10 min)	1.1 ± 0.3	2.9 ± 0.5**	7.4 ± 1.1***	22.9 ± 1.2***	37.9 ± 1.5***
Amplitude (cm H_2O)	0.2 ± 0.04	0.2 ± 0.03	0.3 ± 0.03	0.5 ± 0.06**	1.5 ± 0.25***
Intragastric pressure (cm H_2O)	− 0.5 ± 0.11	− 0.4 ± 0.12	− 0.1 ± 0.11*	0.2 ± 0.13**	0.6 ± 0.16***
B) Rats drinking nicotine 25 µg/ml tap water (n = 10)					
Frequency (contractions/10 min)	1.4 ± 0.4	5.0 ± 0.9** +	9.8 ± 1.1***	23.1 ± 2.1***	37.5 ± 3.0***
Amplitude (cm H_2O)	0.1 ± 0.03	0.2 ± 0.03*	0.3 ± 0.03***	0.4 ± 0.04***	1.2 ± 0.16***
Intragastric pressure (cm H_2O)	− 0.4 ± 0.15	− 0.3 ± 0.12	− 0.1 ± 0.08*	0.2 ± 0.05**	0.4 ± 0.12***
C) Rats drinking nicotine 50 µg/ml tap water (n = 11)					
Frequency (contractions/10 min)	1.0 ± 0.4	5.3 ± 1.1** +	11.4 ± 1.6*** +	30.1 ± 3.2*** +	43.7 ± 3.7***
Amplitude (cm H_2O)	0.1 ± 0.03	0.2 ± 0.02	0.03 ± 0.04*	0.4 ± 0.06**	1.2 ± 0.14***
Intragastric pressure (cm H_2O)	− 0.5 ± 0.21	− 0.4 ± 0.19	− 0.3 ± 0.16*	0.1 ± 0.15**	0.6 ± 0.2***

Values are the means ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when compared to its own vehicle-injected control. + $p < 0.05$ when compared to the corresponding bethanechol-injected group in A.

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Deficits in social behavior in autism and their modification by a synthetic adrenocorticotrophic hormone (4-9) analog

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Abstract. When charting the structure of the social behavior of autistic children by means of an ethologically analyzed playroom session, deficits appeared in the reciprocity of eye-contact and in the location of verbal initiatives. These deficits in social behavior were beneficially influenced by treatment with the adrenocorticotrophic hormone (4-9) analog ORG 2766.
Key words. Social behavior; neuropeptides; ethology; autism.

Many important activities that people engage in take place in social settings. The quality of social functioning and the profits of social interaction contribute to a large extent to a feeling of well-being and determine the level of adaptation of an individual. Conversely, many psychiatric disorders are characterized by disturbed social behavior and associated distress.

Autism is a chronic persisting psychiatric disorder that is preeminently marked by deficits in social interaction. In addition, autistic children are characterized by repetitive stereotyped behavior patterns and a deviant development of cognitive, language and communicative functions [1, 2]. Focussing on disturbances in social behavior, it is not so much differences in frequencies of occurrence of particular behaviors, but rather the differences in the quality of social behavior that are of interest. The quality of social behavior refers to aspects of mutuality and reciprocity and to the structure of behavior. Ethologists have developed concepts and methods which enable one to analyze qualitative aspects of behavior in quantitative terms [3, 4].

Therefore, in a first study we applied ethological methods for the observation and analysis of behavior to chart the deficits in the structure of social behavior of autistic children. All subjects were included in the study only after the nature and the consequences of the procedures had been fully explained to them and their parents, and informed consent was obtained. The concept of the structure of behavior refers to the tempero-sequential relationships of various behavior elements and to the distinction of behavior systems [3, 4]. This process of pattern detection is based, among others, on the temporal contingencies of behavior elements.

The social behavior of 14 autistic children, and by way of contrast, of 10 nonautistic retarded control children was observed in a semistructured playroom session lasting 20 min. The diagnosis of autism had been made independently by two child psychiatrists according to DSM-III-R criteria (1) on the basis of extensive diagnostic evaluations, which included a review of prior records, a parent interview, a child-psychiatric observation and a complete medical diagnostic work-up. All children met the DSM-III-R criteria for autistic disorder (299.00). Age of the autistic children was 8.7 ± 2.1 years (mean ± SD). Their IQ was 69.1 ± 25.0 (mean ± SD). Controls were nonautistic retarded children matched on age and IQ: age 8.9 ± 1.7 year, IQ 81.7 ± 12.0.

In the playroom session, the child is with an unfamiliar experimenter in a playroom. The experimenter presents the child with some tasks in a fixed sequence. The tasks in this case were consecutively a constructive task (building a gate with wooden blocks), a motor activity task (hopping), a drawing task (drawing a boat on the blackboard) and a musical task (playing a tune on a flute). The time-relationships of single behavior elements are registered by means of an event-recorder. The behavior ele-