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Effects of Nicotinamide on Central Cholinergic Transmission and on Spatial Learning in Rats

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KÖPPEN, A., J. KLEIN, B. H. SCHMIDT, F.-J. VAN DER STAAY AND K. LÖFFELHOLZ. *Effects of nicotinamide on central cholinergic transmission and on spatial learning in rats.* PHARMACOL BIOCHEM BEHAV **53**(4) 783–790, 1996. – High-dose nicotinamide (1000 mg/kg) leads to a minor increase of plasma choline but to a major increase of the choline concentrations in the intra- and extracellular spaces of the brain. In the hippocampus, the nicotinamide-induced increase in choline was associated with an increase in the release of acetylcholine under stimulated conditions. In young rats, nicotinamide in doses between 10 and 1000 mg/kg did not influence spatial learning, as tested in the Morris water maze. In old rats, low doses of nicotinamide were ineffective whereas the high dose of 1000 mg/kg even impaired spatial learning. The combined administration of choline and nicotinamide had a synergistic effect on brain choline levels but had similar effects as nicotinamide given alone in the behavioural experiments. Additional tests for spontaneous behaviour and locomotion revealed procholinergic and sedative effects of the compound. We conclude that the ineffectiveness of the putative cognition enhancer nicotinamide in the learning task may be due to the observed sedative effect. Therefore, the development of nonsedative nicotinamide derivatives is recommended.

Acetylcholine Choline Morris water maze Nicotinamide

THE CORRELATION between the decrease in central cholinergic activity and the severity of cognitive dysfunctions in Alzheimer's disease (6,15) has led to the "cholinergic hypothesis of geriatric memory dysfunctions" (2,5). This hypothesis states that the memory impairments in senile dementia occur because of a decreased central cholinergic transmission and implies that an increase in central cholinergic activity should lead to an improvement of the symptoms. Many clinical studies probed the effect of treating patients suffering from senile dementia with dietary supplements of the acetylcholine precursor choline (or lecithin) to enhance central cholinergic activity. However, most of the studies described disappointing results [review: (21)].

One reason for the failure of precursor therapy is the limited ability of exogenous choline to elevate the extracellular choline level in the brain. In previous investigations we found that the administration of choline chloride to rats led to dose-dependent increases in choline in the arterial plasma and to the uptake of large amounts of choline into the brain. However, this uptake was followed by only a moderate enhancement of extracellular choline in the brain (17,19). In addition to cellular uptake, the rapid outward transport of excess choline from the brain into the venous outflow was identified as a homeostatic mechanism that regulates the choline concentration in the brain extracellular space (16,17). It was therefore of great interest that nicotinamide apparently inhibits the outward transport of choline from the brain (12). Nicotinamide, given to rats in high doses, leads to a long-lasting increase in the choline concentration in the cerebrospinal fluid (12) and in the extracellular space, as measured by microdialysis (19). Nicotinamide is metabolized to *N*-methylnicotinamide in rats and humans (14,27), and *N*-methylnicotinamide is a competi-

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tive inhibitor of the choline transporter responsible for the clearance of choline from the extracellular space of the brain (1,22).

The present experiments were carried out to study the effects of nicotinamide on extracellular choline and acetylcholine release and to characterize the possible behavioural consequences of these biochemical responses. For this purpose, we tested the cognitive functions of nicotinamide-treated rats with respect to spatial learning in the Morris water maze. In addition, we tested the effects of nicotinamide on spontaneous behaviour and motility.

METHODS

Materials

All reagents used were analytical grade. Nicotinamide and choline chloride were purchased from Sigma Chemical Co. (Deisenhofen, Germany).

Choline Levels in Blood and Total Brain

Male Wistar rats (200–300 g body weight; Charles River, Germany) were injected SC with nicotinamide and/or IP with choline chloride as detailed in the Results section. Under pentobarbital anaesthesia (60–80 mg/kg, IP), arterial blood was withdrawn from a peripheral artery (A. femoralis). Subsequently, the animals were killed by a microwave beam (2450 GHz, 3 kW) focused on the head for 5 s. Brain tissue was removed completely and homogenized in 10 volumes of ice-cold 85% acetone/15% 1 M formic acid. The homogenate was centrifuged, and aliquots of the supernatant were evaporated to dryness in a vacuum centrifuge and stored at 4°C. Blood was centrifuged at 800 × *g* at room temperature, and plasma was mixed with twice its volume of ice-cold 96% ethanol and centrifuged for 10 min in the cold. Aliquots of the supernatants were evaporated to dryness in a vacuum centrifuge and stored at 4°C. After the residue was redissolved in HPLC buffer, choline was analysed by HPLC with electrochemical detection combining a cation-exchange column (Nucleosil 5 SA, 60 × 4.6 mm, flow rate 1 ml/min) and a reactor column with immobilized choline oxidase (E.C. 1.1.3.17.) (Biometra, Göttingen, Germany). Hydrogen peroxide formed in the enzyme column was detected with a platinum electrode operating at 0.5 V. The mobile phase was 0.1 M sodium phosphate buffer, pH 7.4, with 20 mM tetramethylammonium chloride. The detection limit of the analytical system was about 1 pmol. The results are given as means ± SEM of number (*N*) of experiments. The effects of nicotinamide and choline chloride on choline levels were assessed by Student's *t*-test.

Choline and Acetylcholine Levels in the Extracellular Space of the Hippocampus

Extracellular concentrations of choline and acetylcholine were determined by microdialysis. The manufacture of the dialysis probes and the surgical procedure used to insert the probe were essentially as described by others (3,29). Briefly, under pentobarbital anaesthesia (60–80 mg/kg, IP), the rats were placed in a stereotactic frame and a self-made concentric, I-shaped microdialysis probe (240 μm o.d.; membrane from Filtral AN-69 HF, Hospal, Meyzieu, France) was implanted into the right ventral hippocampus, using the following coordinates (from lambda): AP +3.5; L −4.6; DV −7.0 mm (28). The dialysis membrane had a cutoff of 10,000 Da and a length of 3 mm. The in vitro recovery of choline was 19.0 ± 1.2% and of acetylcholine 17.6 ± 0.9% (*N* = 3). All experiments were carried out on conscious animals 1 or 2 days after the surgery. The microdialysis probe was perfused at a constant rate of 2 μl/min with Ringer's solution (in mM: NaCl 147; KCl 4; $CaCl_2$ 1.2; $MgCl_2$ 1.2). Neostigmine (10 μM) was added to the perfusion solution for acetylcholine measurements. The perfusate was collected at 15-min intervals and samples were injected without further workup into a HPLC system consisting of a polymer column (530 × 1 mm, flow rate 0.12 ml/min) and an electrochemical detector (BAS; Axel Semrau GmbH, Sprockhövel, Germany). The mobile phase was 29 mM NaH_2PO_4, 22 mM sodium acetate, pH 8.5. The detection limit was 100 fmol. Baseline output of choline and acetylcholine was defined as the average output of three consecutive samples which did not differ by more than 5%. The results are given as extracellular concentrations (in μM ± SEM of *N* of experiments) that were calculated from the perfusate concentrations corrected for recovery. Statistical analysis was done with Student's *t*-test. After completion of the experiments, the brain was dissected out to ascertain the location of the dialysis probe in the hippocampus by visual examination.

Morris Water Maze

Animals. Three-month-old and 24-month-old male Wistar rats (WISW : Bor; Winkelmann, Borchen, Germany) were used for behavioural testing. The animals were thoroughly examined, and only those animals were used that appeared to be healthy and showed no signs of behavioural abnormalities. The young animals were kept in standard type III Makrolon cages (40 × 25 × 15 cm), and the older rats in type IV Makrolon cages (56 × 34 × 20 cm; Ehret, Emmerdingen, Germany). The animals were kept in groups of five under conditions of uniform humidity (50%), temperature (21.5°C), and light (12 L : 12 D cycle) and had ad lib access to food and water. When testing started, all rats were transferred to the experimental room, where they were housed during the entire testing period. The light : dark regimen was the same as in the vivarium.

Apparatus. The maze consisted of a circular black basin with a slightly sloping wall (diameter at top 153 cm, diameter at bottom 143 cm, depth 63 cm) filled with 43.5 cm of clear tap water at a temperature of approximately 22°C. The escape platform was a black polyethylene cylinder (diameter 10.8 cm), submerged 1.5 cm below the surface of the water. Only distal cues (i.e., standard room objects) could be used by the rats for spatial learning and these cues were kept unchanged throughout the testing period. A video camera, mounted in the centre above the pool, provided a picture of the water tank on a monitor. Lines on the monitor defined the quadrant boundaries and the position of the escape platform. Each quadrant was further subdivided by a pattern of lines (a 4 × 4 matrix of squares; corresponding to a distance between grid lines in the pool of 16.9 cm) to obtain an arbitrary measure of the distance swum. Crossing a line was scored when a rat moved across it with its whole body. The movements of the rats were registered manually and the data were stored in a microcomputer.

Procedure. For 5 consecutive days each animal was given four trials per day. One hour prior to the first trial the rats received nicotinamide and/or choline chloride by gavage (5 ml/kg; in tap water). The control animals were treated with tap water. A trial was started by placing a rat in the pool, facing the wall of the tank. Each of the four starting positions was used once in the series of four trials; their order was randomized. The escape platform was always in the same

quadrant. A trial was terminated as soon as the rat had climbed onto the platform or when 90 s had elapsed. In the latter case the animal was put on the platform by the experimenter. Each animal was allowed to stay on the platform for 30 s before the next trial was started. There were nine rats per treatment group.

Data analysis and statistics. Three measures were analysed: 1) the escape latency (26), which is the time the rats needed to reach the submerged platform; 2) the number of line crossings; and 3) the swimming speed (number of line crossings divided by escape latency). The second measure can be taken as an index of the distance swum or, alternatively, as an index of the extent to which a rat explored the water tank. The measures were averaged per rat within each session. Differences in the acquisition of the water escape task were assessed by analysis of variance (ANOVA) (33) with repeated measures over sessions. In addition, differences between the means of particular sessions were analysed by using *t* statistics.

Modified Irwin Test

Male Wistar rats were transferred separately to type II Makrolon cages (22 × 16.5 × 14 cm; Ehret, Emmerdingen, Germany). After 30 min they were treated with nicotinamide or nicotinamide + choline chloride orally (5 ml/kg). During the following hours they were observed every 15 min for signs of depressant, stimulant, and autonomic effects as described by Irwin (11). The proportion of animals showing a given response was recorded.

Spontaneous Locomotor Behaviour

Spontaneous locomotor activity was evaluated in circular activity cages (diameter 24 cm; ZW 41, Laue-Elektromechanik, Elmshorn, Germany). Animal movements along horizontal coordinate axes were measured by means of 19 infrared photobeams and the total number of animal movements (beam interruption) was recorded automatically. Groups of five mice (female NMR, Charles River, Sulzfeld, Germany) were preexposed to the activity cages for 2 min and spontaneous locomotor activity was recorded for 2 min. Subsequently, the mice were treated with saline (IP; control group), choline chloride (20 mg/kg, IP), nicotinamide (0.2–1.2 g/kg, SC), or nicotinamide plus choline chloride, and the second measurements (2 min) were carried out 15 min after saline or choline chloride administration and 2 h after nicotinamide administration. The results are given as percentages of the locomotor activity of the untreated mice.

RESULTS

Influence of Nicotinamide on Choline Concentrations

The effects of nicotinamide (1.2 g/kg, SC; dissolved in water), choline chloride (20 mg/kg, IP; dissolved in saline), and the combined administration of both substances on the choline concentrations in different compartments are shown in Fig. 1. The samples were taken 15 min after choline chloride or 2 h after nicotinamide administration. For evaluation of the combined administration, the rats first received nicotin-

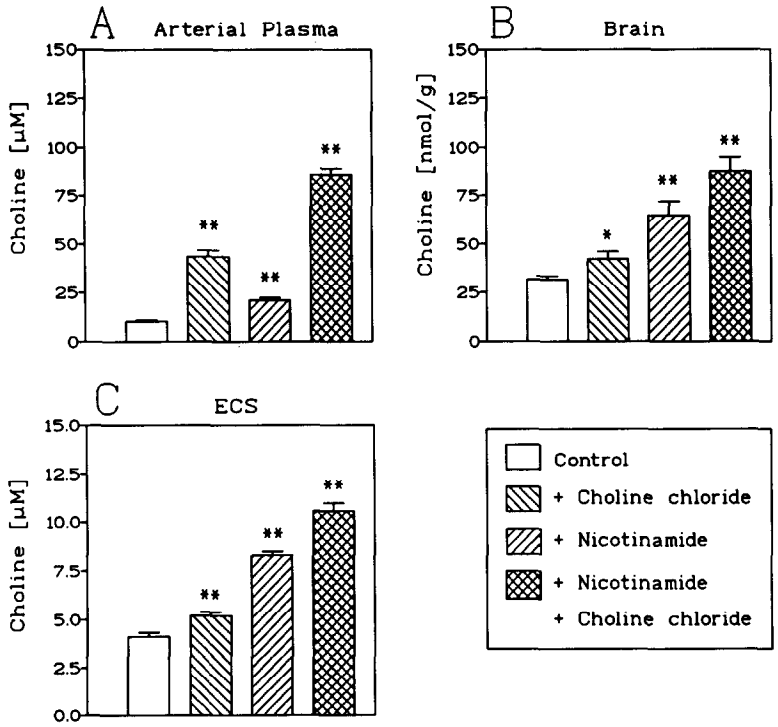

FIG. 1. Choline concentrations in arterial plasma (A), in brain homogenate (B), and in the extracellular space (ECS) of the hippocampus (C) under different conditions. Samples were taken 2 h after the administration of nicotinamide (1.2 g/kg, SC) or 15 min after the injection of choline chloride (20 mg/kg, IP). Results are given as means ± SEM of 5 to 12 experiments. $*p < 0.02$; $**p < 0.01$.

amide and then choline chloride 2 h later; the samples were taken 15 min after the second injection. Maximal effects were observed at these time points in a previous study (19). All treatments significantly increased the arterial plasma choline concentration (Fig. 1A; basal value: 10.3 ± 0.6 μM, $N = 12$) and the choline concentration in the brain homogenate (Fig. 1B; basal value: 31.4 ± 1.9 nmol/g, $N = 12$), which mainly represents the *intracellular* choline concentration of the brain. Exogenous choline increased the arterial choline concentration more than the brain choline concentration, whereas nicotinamide had the opposite effect (brain choline > arterial choline). A similar pattern was obtained when the choline levels in the *extracellular* space of the hippocampus were analysed by microdialysis (Fig. 1C). The basal efflux of choline into the hippocampal dialysate was 1.56 ± 0.08 pmol/min ($N = 6$). After correction for in vitro recovery this corresponds to a concentration in the extracellular space of 4.11 μM. This basal level was slightly, but significantly, increased by 26% in response to choline administration but was increased by 102% in response to nicotinamide. The value was further increased to 157% ($p < 0.01$ vs. nicotinamide alone) by the combined administration of nicotinamide and choline.

Influence of Nicotinamide on Acetylcholine Release

In the presence of 10 μM neostigmine in the perfusion fluid, the basal acetylcholine efflux from the hippocampus amounted to 0.12 ± 0.01 pmol/min ($N = 6$), from which an acetylcholine concentration in the extracellular space of 0.34 ± 0.03 μM (Fig. 2) can be estimated. The administration of the muscarinic antagonist atropine (5 mg/kg, IP) increased the acetylcholine release by 125% compared to that of control animals 60 min after injection. However, when the rats were first treated with nicotinamide and subsequently (after 2 h) with atropine, a maximal increase in acetylcholine of 268% was observed. Thus, nicotinamide pretreatment more than doubled ($p < 0.02$) the acetylcholine efflux in the presence of atropine. When the animals were first treated with nicotinamide and then with atropine plus choline chloride 2 h later, the stimulated acetylcholine release (by 267% of control; $p < 0.01$) was equal to that of the animals treated with nicotinamide alone. Thus, exogenous choline had no additional effect.

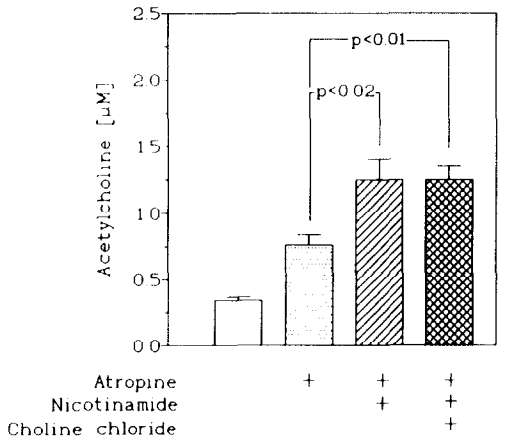

FIG. 2. Acetylcholine concentrations in the extracellular space of the hippocampus in awake rats under basal conditions (open bar) and under stimulated conditions. Acetylcholine release was stimulated by injection of atropine (5 mg/kg, IP), and samples were taken from the microdialysate 60 min after atropine administration. Nicotinamide (1.2 g/kg, SC) was administered 2 h before the atropine injection; choline chloride (20 mg/kg, IP) was given together with atropine. Results are expressed as the mean concentration of acetylcholine in the extracellular space (calculated from the concentrations in the microdialysate after correction for recovery) ± SEM of five to six experiments.

Effects of Nicotinamide on Spatial Learning

The cognitive abilities of the animals after oral administration of nicotinamide alone or after nicotinamide in combination with choline chloride were tested in the Morris water maze, a spatial learning paradigm. For this purpose the test compounds were administered 1 h before testing by gavage to avoid stressful IP or SC administration procedures. Nicotinamide is well absorbed after PO administration (10). Peroral administration of nicotinamide increased extracellular choline to a concentration comparable to that measured after SC administration of nicotinamide (PO administration: 205% of control; SC administration: 202% of control). Figure 3 depicts the effects of different doses of nicotinamide (100, 300, and 1000 mg/kg, PO) given in combination with choline chloride (20 mg/kg, PO) in young rats.

The *escape latency* (i.e., the time taken to find and escape onto the submerged platform) decreased over sessions, $F(4, 128) = 57.66$, $p < 0.01$ (Fig. 3A). The learning curves, however, were not affected by the administration of nicotinamide or choline chloride [treatment, $F(3, 32) = 1.96$, NS; treatment by session interaction, $F(12, 128) < 1.0$, NS]. The same is true for the *number of line crossings* (i.e., the distance swum to reach the submerged platform). Over sessions, the rats learned to reduce the distance swum to find the platform, $F(4, 128) = 50.04$, $p < 0.01$ (Fig. 3B). Again, treatment with the test substances had no effect on the learning curves [$F(3, 32) = 1.95$, NS; treatment by session interaction, $F(12, 128) < 1.0$, NS]. The *swimming speed* slightly increased over sessions, $F(4, 128) = 10.90$, $p < 0.01$ (Fig. 3C), but was not differentially affected by the test substances [treatment, $F(3, 32) < 1.0$, NS; treatment by session interaction, $F(3, 32) < 1.0$, NS].

Very similar results were found after the administration of nicotinamide alone (results not shown). In a further series of experiments we used lower doses of nicotinamide in combination with choline to test a possible biphasic effect of nicotinamide. Nicotinamide in doses of 10 and 30 mg/kg, each in combination with choline chloride at a concentration of 20 mg/kg, did not affect the learning performance of rats in the Morris water escape task (results not shown).

As young rats acquire the Morris water maze task very quickly, subtle cognition-enhancing effects might not have been demonstrable because of floor effects. Therefore, we performed an additional experiment with 24-month-old rats (Fig. 4). Previous studies have demonstrated that rats of this age show impaired acquisition of the Morris task when compared to young (e.g., 3-month-old) animals (24,30). The different treatment groups received nicotinamide (100 and 1000 mg/kg, PO), each in combination with choline chloride (20 mg/kg), or choline chloride alone.

The treatments affected the decrease in the *escape latency* over sessions differently [treatment by session interaction, $F(4, 120) = 2.11$, $p < 0.05$; Fig. 4A]. As 80% of the variation of the decrease in the escape latency over sessions is explained by the linear component of the learning curves, the

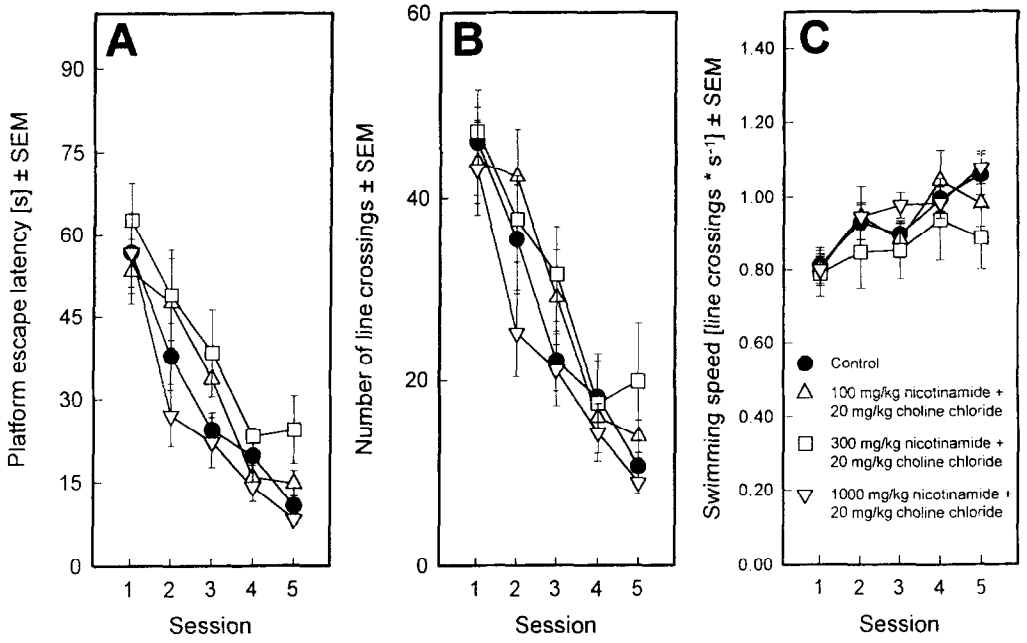

FIG. 3. Effects of different doses of nicotinamide (PO), each in combination with choline chloride, on escape latency (A), number of line crossings (B), and swimming speed (C) of young (3-month-old) rats in the Morris water maze. The control animals received tap water. Results are given as mean ± SEM for nine rats.

linear trend components of the treatment groups were compared by post hoc analysis (Least Significant Difference). This analysis confirmed that the rats treated with the combination of 1000 mg/kg nicotinamide and 20 mg/kg choline chloride had a flatter learning curve than the controls did, whereas the learning curves of the groups that received choline chloride alone or the combination of 100 mg/kg nicotinamide and choline chloride did not deviate from those of the controls.

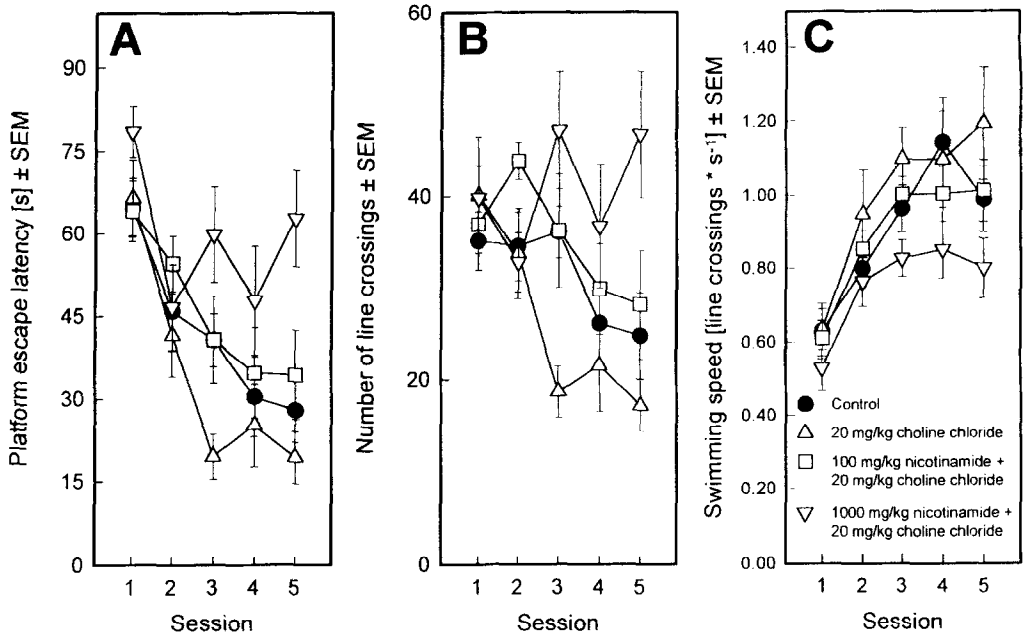

FIG. 4. Effects of different doses of nicotinamide (PO), each in combination with choline chloride, and choline chloride alone on escape latency (A), number of line crossings (B), and swimming speed (C) of 24-month-old rats in the Morris water maze. The control animals received tap water. Results are given as mean ± SEM for eight to nine rats.

TABLE 1

EFFECT OF NICOTINAMIDE AND NICOTINAMIDE IN COMBINATION WITH CHOLINE CHLORIDE ON SPONTANEOUS BEHAVIOUR OF RATS

	Nicotinamide (1000 mg/kg)		Nicotinamide (1000 mg/kg) + Choline Chloride (20 mg/kg)	
Time (min)	Ptosis	Salivation	Ptosis	Salivation
15	0	2	0	2
30	0	4	0	3
45	0	4	3	4
60	2	4	3	4
75	2	2	3	3
90	0	0	0	0

Observed symptoms in rats after treatment (PO) with nicotinamide or nicotinamide in combination with choline chloride. All symptoms observed are presented and are given for six rats at different times after treatment. After 90 min all animals were free of symptoms.

Virtually the same result was found for the *number of line crossings* (i.e., the distance swum to reach the submerged platform) (Fig. 4B). The *swimming speed* was not affected by administration of the testing substances (Fig. 4C). However, a marginal prolongation of the swimming speed was observed in rats treated with the high dose of nicotinamide, $F(3, 30) = 2.62$, $0.1 > p > 0.05$.

Effects of Nicotinamide on Spontaneous Behaviour

In a modified Irwin test [(11); Table 1] nicotinamide did not produce detectable effects in young male rats in doses of up to 300 mg/kg PO. At the high dose of 1000 mg/kg a brief period of ptosis and salivation was observed. All animals showed signs of sedation. There were no additional effects of choline chloride (20 mg/kg, PO).

From reasons of economy, the sedative response was studied further in mice. As depicted in Fig. 5, choline chloride (20 mg/kg, IP) had no effect on the motor activity of mice. However, nicotinamide led to a dose-dependent reduction of motor activity that was not influenced by additional choline chloride administration.

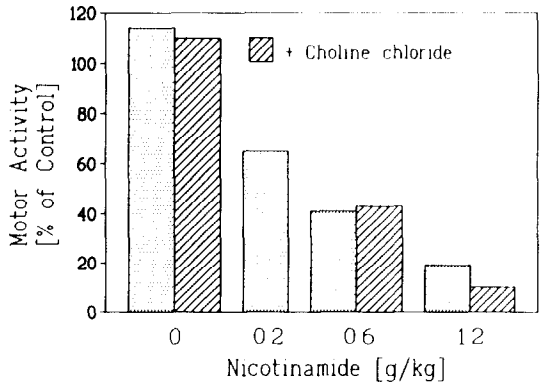

FIG. 5. Effects of different doses of nicotinamide (SC) alone and in combination with choline chloride (20 mg/kg, IP) on the spontaneous locomotor behaviour of mice. Locomotor activity was measured in an activity cage 15 min after choline chloride administration and 2 h after nicotinamide administration.

DISCUSSION

Effects of Nicotinamide on Choline Concentrations and Acetylcholine Release

The development of drugs that enhance central cholinergic transmission is a major objective of current pharmacological research. The present study suggests that high-dose nicotinamide is a useful model drug for this purpose because nicotinamide raises the levels of choline and increases the release of acetylcholine in the brain. The neurochemical data summarized in Fig. 1 demonstrate that the administration of nicotinamide is a rather selective way to increase brain choline levels. Nicotinamide led to a moderate increase in plasma choline but to a pronounced increase in total brain choline (Fig. 1), as reflected by the doubling of the choline levels in the intracellular (Fig. 1B) as well as the extracellular (Fig. 1C) space. In contrast, choline chloride (20 mg/kg) was found to raise choline levels in the blood by more than fourfold but the increase in brain choline was limited to 25–30%. Moreover, we recently demonstrated that nicotinamide leads to an increase in brain choline that is much longer lasting than that caused by exogenous choline (19). One reason for the limited influence of exogenous choline on brain choline levels is the rapid release of excess choline from the brain into venous blood (16–18). The inhibition of this release is probably responsible for the selective effects of nicotinamide on brain choline (12,22).

In addition to changes in choline levels, we also determined the effects of nicotinamide on the release of acetylcholine (ACh) from cholinergic nerve endings in the hippocampus by using the microdialysis technique. Pretreatment of the animals with nicotinamide did not increase the basal efflux of ACh in the microdialysate (data not shown), in spite of concomitant increases in choline (cf. Fig. 1C). However, clear-cut effects were noted if the animals were injected with atropine, a muscarinic antagonist that increases ACh release by the blockade of inhibitory presynaptic receptors. Whereas atropine alone increased ACh efflux by 125% of control, pretreatment with nicotinamide increased the stimulated ACh efflux by 268% of control. This study provides evidence, for the first time, that a drug-induced increase in the availability of free choline in the brain in vivo leads to a marked acceleration of ACh release in the presence of atropine. Thus, a facilitation of central cholinergic transmission by nicotinamide can be expected in situations in which cholinergic pathways are particularly ac-

tive (e.g., during learning tasks). Moreover, previous microdialysis studies showed that the effects of choline administration on ACh release in the CNS could only be observed when choline was given locally in large doses (20), but not after peripheral administration [(32), review: (31)].

Finally, the combined administration of nicotinamide and choline revealed synergistic effects of these substances on choline levels in the blood and brain (Fig. 1). However, additional choline administration did not increase the stimulated ACh release further in animals that had previously received nicotinamide (Fig. 2). Thus, it appears that an elevation of the extracellular choline level to values beyond 8 μM (Fig. 1C) does not further enhance ACh synthesis. The reason for this observation may be saturation of high-affinity choline uptake into cholinergic presynaptic terminals (13).

Effects of Nicotinamide on Behavioural Parameters

The crucial role of the central cholinergic system for memory and learning has been confirmed in a wide range of behavioural studies since the original concept was proposed by Deutsch (7). The Morris water maze (26) is one of the most popular paradigms for testing learning and memory and was therefore chosen for the present study on the influence of high-dose nicotinamide on cognitive functions. In this task, the rats learn to locate a submerged platform by using distal cues; thus, the task measures predominantly spatial reference memory. Previous studies have confirmed that the outcome measures of the Morris water maze are sensitive to cholinergic manipulations. In young rats, cholinergic antagonists or the selective destruction of cholinergic pathways innervating the hippocampus and cerebral cortex leads to a marked reduction in the escape latency, an effect that can be attenuated by esterase inhibitors such as physostigmine [review: (23)]. In the present experiments, the observed increase in nicotinamide-stimulated ACh release from the hippocampus (Fig. 2) was not accompanied by cognitive improvements. In fact, none of the doses of nicotinamide (10–1000 mg/kg), given alone or in combination with choline chloride (20 mg/kg), reduced the latencies of young rats to locate the hidden platform (Fig. 3). A negative influence of nicotinamide on the learning test was also not observed in these animals. Although nicotinamide did not seem to influence learning and memory in this particular task, the lack of effect of nicotinamide may also be the result of two opposing effects (see below).

The data in Fig. 3 demonstrate that the young rats acquired the escape task of the water maze very rapidly. These conditions are not favourable to show possible subtle positive effects of nicotinamide. We therefore repeated the experiments with 24-month-old rats that had been previously found to have a poorer performance (24,30). Previous studies demonstrated that the poor performance of old rats in the water maze can be improved by muscarinic agonists (4) or by cholinergic grafts in the hippocampus (9). However, 100 mg/kg nicotinamide, in combination with 20 mg/kg choline chloride, did not have a significant effect on the measured parameters. Moreover, the high dose of 1000 mg/kg nicotinamide even caused a significant reduction in spatial learning, as demonstrated by a prolongation of the escape latencies and path lengths (Fig. 4).

A possible explanation for the lack of effect of nicotinamide in the learning task is the sedative action observed in the present study. Nicotinamide caused a drastic, dose-dependent reduction in the spontaneous motility of mice (Fig. 5). At 1.2 g/kg nicotinamide, the spontaneous motility of the mice was less than 20% of that of the controls within 2 h; however, motor coordination (measured by rotarod) was not impaired by nicotinamide treatment (data not shown). Möhler et al. (25) reported that nicotinamide in high concentrations shared common actions with benzodiazepines in electrophysiological and behavioural experiments, suggesting a role for nicotinamide as an agonist at the benzodiazepine-GABA-receptor complex. GABA agonists repeatedly have been shown to impair the performance of rats in the Morris water maze (23). As benzodiazepine-like drugs do not influence the brain choline concentration (8), the possible action of nicotinamide on GABA receptors cannot be responsible for the observed increase in brain choline.

Taken together, these data suggest that nicotinamide increased the availability of free choline by a central effect, which in turn enhanced the evoked ACh release. However, nicotinamide did not improve learning and memory, as determined in the Morris water maze, possibly because of a sedative effect of the drug. The development of nicotinamide derivatives with a more favourable spectrum of side effects may be a useful approach to the therapy of central cholinergic dysfunctions.

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