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Behavioral effects of the α_2-adrenoceptor antagonist, atipamezole, after focal cerebral ischemia in rats

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Abstract

The present study characterized the behavioral effects of the selective α_2-adrenoceptor antagonist, atipamezole, in a rat model of focal cerebral ischemia. Atipamezole (1 mg/kg, s.c.) or desipramine (5 mg/kg, i.p.), a noradrenaline reuptake blocker, was administered either as a single injection 2 days after ischemia induction or for 10 days thereafter (subacute administration). A subacute atipamezole treatment given 30 min before behavioral assessment improved performance in the limb-placing test (days 5, 7, 9, and 11) and in the foot-slip test (days 3 and 7), but not in the beam-walking test. There was no difference between experimental groups in behavioral performance following a single administration of atipamezole or following single or subacute administration of desipramine. The drug treatments did not attenuate the impairment of spatial cognitive performance of ischemic rats in the Morris water-maze test. These results suggest that repeated use-dependent release of noradrenaline by atipamezole facilitates the sensorimotor recovery following focal cerebral ischemia in rats.

Keywords: α_2-Adrenoceptor antagonist; Focal cerebral ischemia; Recovery; Sensorimotor functions; (Rat)

1. Introduction

Despite extensive research, there is no effective treatment available to prevent or restrict acute neuronal damage after stroke (Fisher, 1999). Most stroke patients, however, exhibit some degree of spontaneous functional recovery after the initial disabling state (Nakayama et al., 1994). This recovery process can be enhanced by physical therapy or possibly more effectively by combining physical therapy and drug treatment (Feeney, 1998). Combination of both physical therapy and pharmacotherapy is clinically appealing, because functional recovery might be facilitated even when treatment is initiated days or weeks after the ischemic insult (Sutton et al., 1989). This is in contrast to pharmacotherapy of acute ischemic stroke, which should be initiated within a few hours after initial signs of brain injury (Baron et al., 1995).

Pharmacologic manipulation of the recovery process following brain trauma stems back to the 1940s when Mailing and Acheson (1946) demonstrated that decorticate cats regained their lost placing reflexes after amphetamine administration. The recovery-enhancing effect of amphetamine has since been shown in several experimental models, including sensorimotor cortex ablation (Feeney et al., 1982), contusive brain injury (Feeney and Sutton, 1988), and focal cerebral ischemia (Hurwitz et al., 1991). Preliminary clinical studies also indicate a positive treatment effect in a small number of stroke patients (Crisostomo et al., 1988; Walker-Batson et al., 1995). Increased noradrenergic activity appears to underlie the facilitatory effect of amphetamine on recovery, because intraventricular administration of noradrenaline, but not dopamine or serotonin, facilitates recovery after sensorimotor cortex lesions (Boyeson and Feeney, 1989). Classical α_2-adrenoceptor antagonists, idazoxan or yohimbine, also facilitate recovery following cerebral lesions (Goldstein et al., 1989; Sutton and Feeney, 1992), possibly by

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blocking α_2-autoreceptors in the locus coeruleus, which in turn increases the firing rate of the ascending noradrenaline-containing neurons (Simson and Weiss, 1987). This, together with a blockade of presynaptic α_2-autoreceptors, increases the release of norepinephrine in response to physiologic stimuli.

Most of the evidence of the recovery-facilitating effects of noradrenergic compounds comes from studies using cortical ablations followed by assessment of functional recovery by the beam-walking test (Feeney, 1998). We recently demonstrated that stimulation of the noradrenergic system by the selective α_2-adrenoceptor antagonist, atipamezole, improved performance of rats in sensorimotor tests following focal cerebral ischemia (Puurunen et al., 1997b). Compared to idazoxan or yohimbine, atipamezole has an approximately 100 times greater affinity for α_2-adrenoceptors, it has no affinity for 5-HT_{1A} receptors, and its affinity for the imidazole binding sites is negligible (Virtanen et al., 1989; Haapalinna et al., 1997). The selectivity and safety over a wide dosage range makes atipamezole an ideal tool to study the role of the noradrenergic system in the recovery process following experimental stroke.

The present study further characterized behavioral effects of atipamezole in a rat model of focal cerebral ischemia. A combination of behavioral tests was used to measure the outcome following 10 daily administrations of atipamezole (referred to in the text as subacute administration) or a single administration of atipamezole. The rats were tested 30 min after drug administration because the action of noradrenergic compounds is suggested to be dependent on task-relevant practice during the period of drug action (Feeney et al., 1982). In addition, the rats were housed in an enriched environment starting on day 2 to provide additional sensorimotor stimulation and exercise. Environmental enrichment improves recovery of sensorimotor deficits in rats following ischemia (Ohlsson and Johansson, 1995). The results are compared to those obtained following administration of desipramine, a noradrenaline reuptake blocker, which facilitates recovery following lesion to the sensorimotor cortex (Boyeson and Harmon, 1993).

2. Materials and methods

2.1. Focal ischemia model

The experimental procedure was approved by the Committee for the Welfare of Laboratory Animals of the University of Kuopio and by the Provincial Government of Kuopio. Male Wistar rats (275–315 g) were used for the study. Focal cerebral ischemia was induced using the intraluminal filament technique (Jolkkonen et al., 1999). Anesthesia was induced with 3% halothane in 30% O_2/70% N_2O and then maintained throughout the operation with 0.5% to 0.6% halothane delivered via a nose mask. The right common carotid artery was exposed through a midline cervical incision. The heparinized intraluminal filament (Ø 0.28 mm, rounded tip) was introduced via the external carotid artery, 1.9 to 2.1 mm into the internal carotid artery to occlude the blood flow to the middle cerebral artery territory. The rectal temperature was monitored and maintained at 37°C using a heating pad (Harvard Homeothermic Blanket Control Unit, 50-7061). After 120 min of occlusion, the filament was gently pulled out and the external carotid artery was permanently closed by cauterization. In sham-operated rats, the right common carotid artery was exposed and the external carotid artery was electrocoagulated without introducing the filament into the internal carotid artery. After the operation, the animals were allowed to wake up in an incubator (30°C) and were then moved to their home cages. Animals were given supplemental 0.9% NaCl (i.p.) during the first postoperative days. Blood gasses and blood pressure were not monitored in the present study, because closing of the femoral artery after blood sampling might have interfered with the performance of rats in the behavioral tests. These physiologic parameters have been shown, however, to be stable during the described procedures (Jolkkonen et al., 1999).

2.2. Study design and drug treatment

Two days after the operation, a modified version of the limb-placing test (De Ryck et al., 1989) was used to assess the behavioral deficit of the animals and to assign them to behaviorally equal experimental groups. Thereafter, the animals were housed in an enriched environment that consisted of two large cages ($61 \times 46 \times 46$ cm) connected together by a short tunnel (Puurunen et al., 1997a). The cages contained tunnels, shelves, a small running-wheel, and different kinds of manipulable objects. The rats were housed in the enriched environment in groups of eight. Housing in the enriched environment provided the rats social contact, sensorimotor stimulation, and spatial exercise.

Study design and timing of drug treatment are shown in Fig. 1. Atipamezole hydrochloride (Orion) and desipramine (RBI) were dissolved in 0.9% NaCl and administered at a dose of 1 mg/kg (s.c.) and 5 mg/kg (i.p.), respectively. The drug doses were selected on the basis of previous studies (Barturen and Garcia-Sevilla, 1992; Puurunen et al., 1997a,b). Our preliminary experiments indicated that the lowest atipamezole dose needed for a significant behavioral effect in the limb-placing task is 1 mg/kg and that desipramine at a dose of 10 mg/kg causes immobility (data not shown). In the first experiment, we examined the effect of subacute atipamezole or desipramine treatment starting on the second day after ischemia induction and continuing for 10 days. Following the first drug injection, the limb-placing and beam-walking

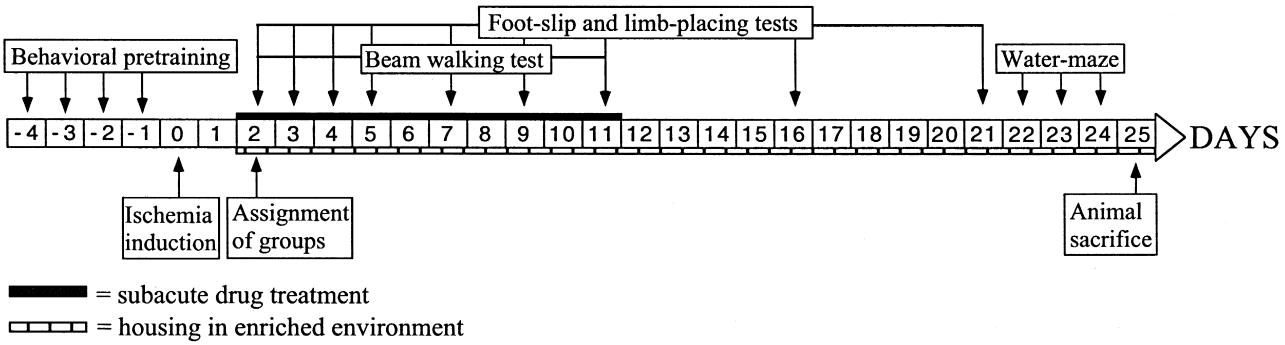

Fig. 1. The study design.

test were performed (30, 60, and 90 min after injection) to determine if there were acute drug effects. All behavioral assessments were scored in a blind manner. In the second experiment, we examined the behavioral effect of a single dose of atipamezole or desipramine administered on the second day after ischemia induction (0.9% NaCl was given for the next 9 days). The ischemic controls were injected with 0.9% saline (s.c. or i.p.). Sham-operated control rats were treated with either 0.9% NaCl, atipamezole or desipramine.

2.3. Behavioral tests

2.3.1. Limb-placing test

This test was a modified version of a test described by De Ryck et al. (1989). The rats were habituated for handling and testing before ischemia induction. The limb-placing test was used to study the recovery on post-operative days 2, 3, 4, 5, 7, 9, 11, 16, and 21 (Fig. 1). The test had seven limb-placing tasks that assess the sensorimotor integration of forelimb and hindlimb responses to tactile and proprioceptive stimulation. The tasks were scored in the following manner: the rat performed normally, 2 points; the rat performed with a delay (>2 s) and/or incompletely, 1 point; and the rat did not perform normally, 0 point. Both sides of the body were tested. In the first task, the rat was slowly lowered toward a table top. Normal rats will stretch both of their forelimbs towards the table while held 10 cm above it. In the second task, the rat was positioned towards the table and its forelimbs were placed on the table. Each forelimb was gently pulled down and retrieval and placement were checked. Normal rats will replace their limbs on the table. The third task was the same as the second one except that by keeping its head upward in a 45° angle, the rat was prohibited from seeing the table or contacting it with its vibrissae. Next, the rats were placed along the table edge to check for lateral placement of the forelimb (fourth task) and the hindlimb (fifth task). In the sixth task, the rat was again positioned towards the table with the hindlimbs just over the table edge. Each hindlimb was pulled down and gently stimulated by pushing towards the side of the table. In the seventh task, the forelimbs of the rat were on the edge of the table and the rat was gently pushed from behind toward the edge. Normal rats resist the pushing, but ischemic rats cannot keep their grip and the affected limb slips off the edge. Total scores for sham-operated rats were 14.

2.3.2. Beam-walking test

The beam-walking test was used to assess deficits in coordination and integration of motor movement, especially in the hindlimb. The rats were pretrained to traverse the beam for 3 days before ischemia induction and all rats had learned the task by the end of the training period. The animals were tested from days 2 to 11 after ischemia induction (Fig. 1). The beam-walking apparatus consisted of a square beam (2.5 cm wide, 122 cm long, at 42 cm high) connected to a black box ($20.5 \times 25 \times 25$ cm). A bright light was placed above the start point to motivate the rats to traverse the beam. A modified rating scale by Feeney et al. (1982) was used to assess the performance of the rats: the rat was not able to stay on the beam, 0 point; the rat did not move, but was able to stay on the beam, 1 point; the rat tried to traverse the beam, but fell, 2 points; the rat traversed the beam with more than 50% footslips of the impaired contralateral hindlimb, 3 points; the rat traversed the beam with more than one footslip, but less than 50%, 4 points; the rat had only one slip of the hindlimb, 5 points; the rat traversed the beam without any slip of the hindlimb, 6 points. Total scores for sham-operated rats were 5.8–6.0.

2.3.3. Foot-slip test

This test measures accurate placing of the impaired contralateral forelimb on the rungs of a motorized wheel. The rats were pretrained to run in a wheel for 4 days before ischemia induction and all rats included in the study learned to run in the wheel. The accuracy of forelimb-placement was quantified using the foot-slip test on days 2, 3, 4, 5, 7, 9, 11, 16, and 21 after induction of ischemia (Fig. 1). The running-wheel (Ø 29 cm, with transparent plastic walls, rungs 2 cm apart) had an adjustable motor and rotated six times per minute. Performance was recorded

by a camera connected to a video recorder and a monitor. Later, the performance of the rats was assessed from video tapes by counting the number of slips and steps taken during the 2-min monitoring period. The total number of slips included slips of the forelimb from a rung and misplacements between rungs. The slip ratio of the impaired contralateral forelimb (number of slips/number of steps taken) was calculated to correct for possible changes in overall motor activity due to drug treatment or uncooperation of the rats. The slip ratio (%) for sham-operated rats was 3.1–5.5%.

2.3.4. Water-maze test

To assess spatial learning, a modified version of the Morris water-maze task was used. Rats were given five trials from the first through the third test days (days 22–24 after ischemia induction) (Fig. 1). The water-maze apparatus was previously described in detail (Riekkinen et al., 1990). The pool was divided into four quadrants of equal surface area. The starting locations were called north, south, east, and west, and were located arbitrarily at equal distances on the pool rim. The platform was located in the middle of the south–west quadrant 25 cm from the pool rim. The swim paths were monitored by a video camera connected to a computer through an image analyzer (HVS image). If the rat failed to find the hidden platform within 70 s, it was placed on the platform. The animal was allowed to remain on the platform for 10 s and to rest for either 30 s (after trials 1–2, 4) or 1 min (after trials 3, and 5 of the third day). The first, third, and fourth trials of the day were started from one of the points located farthest from the platform. The start point was changed after each trial. Escape latency (time to reach the platform) and path length the animal swam to find the platform were used to assess acquisition of the water-maze task. Swimming speed (path length/escape latency) was used to assess the motoric activity of rats in this task.

2.4. Histology

The animals were decapitated 25 days after occlusion of the middle cerebral artery and their brains were rapidly removed from the skull and frozen on dry ice. Coronal sections (40 μm) were cut throughout the brain on a cryostat and sections at 1.0-mm intervals were collected on slides. Sections were stained for 20 min with a solution containing 1.2 mmol/l nitroblue tetrazolium and 0.1 mol/l sodium succinate in 0.1 mol/l sodium phosphate buffer, pH 7.6, at 37°C (Nachlas et al., 1957). Sections were then rinsed in water, dehydrated in an ascending series of alcohol, cleared in xylenes, and cover-slipped with Depex. Estimations of the infarcted areas in the cortex and striatum were performed using an image analysis system (MCID). The area of infarction was determined according to the indirect method of Swanson et al. (1990) by an observer blind to the experimental groups. The image of each section was stored as a 1280×1024 matrix of calibrated pixel units. The digitized image was then displayed on a video screen, areas of interest were outlined separately for each hemisphere, and surviving gray matter with optical densities above the threshold levels were recognized automatically by the image analysis system. The difference between the size of an intact area in the contralateral hemisphere and its respective residual area in the ipsilateral hemisphere was used as the infarcted area. Total infarct volume was calculated by multiplying infarct area by the distance between the sections and summing together the volumes for each brain.

2.5. Statistics

Differences in the infarct volumes between ischemic saline controls and drug-treated rats were analyzed using one-way analysis of variance (ANOVA). Correlations between the infarct volumes and limb-placing deficit on the second day after ischemia induction were determined using Spearman's correlation coefficient. Statistical differences in beam-walking and limb-placing tests between ischemic saline controls and drug-treated ischemic rats were analyzed using the Mann–Whitney *U*-test. Foot-slip data comparisons between groups were made using one-way ANOVA with Duncan's post hoc test. The water-maze data (escape latency, path length, swimming speed) were analyzed using ANOVA for repeated measures.

3. Results

3.1. Infarct volumes

A transient occlusion of the middle cerebral artery in rats produced consistent and extensive cortical and striatal infarction and partial damage to the hypothalamus and amygdala, leading eventually to a fluid-filled cyst (Fig. 2). The typical infarct volumes in the cortex and striatum determined from nitroblue tetrazolium-stained sections were 99.2 ± 43.9 and 34.5 ± 8.7 mm^3 for ischemic saline controls ($n = 13$), respectively. The infarct volumes in the cortex ($r = -0.446$, $P < 0.01$, $n = 42$) but not in the striatum ($r = -0.296$, $P = 0.057$, $n = 42$) correlated with the limb-placing deficit, assessed 2 days after ischemia induction. There were no statistically significant differences in infarct volumes between the ischemic saline controls and drug-treated ischemic rats 25 days after the induction of ischemia.

3.2. Effects of subacute administration of atipamezole

The behavioral performance of ischemic saline controls recovered spontaneously within 2 to 3 weeks to the level of sham-operated rats. Performance in the limb-placing test or beam-walking test did not differ between experimental

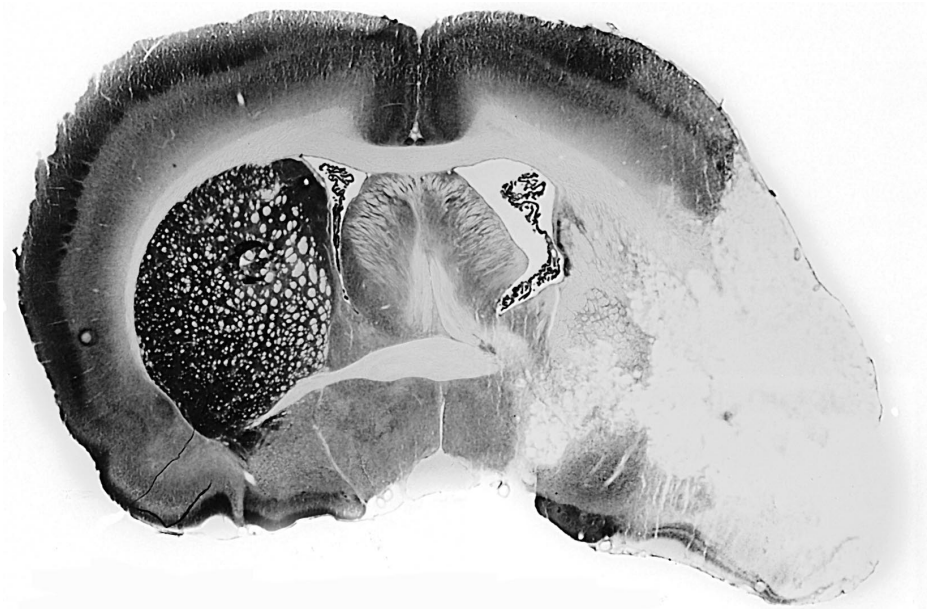

Fig. 2. A brightfield photograph of a coronal section stained with nitroblue tetrazolium showing typical cortical and striatal damage 25 days after transient occlusion of the middle cerebral artery in rats. Note the clear delineation of intact tissue from the liquid-filled cyst.

groups during the first 90 min after the first injections of atipamezole (data not shown). Later, subacute treatment with atipamezole administered 30 min before behavioral assessment improved the total score in the limb-placing task on days 5, 7, 9, and 11 after ischemia induction (Fig. 3A). The drug effect was particularly obvious in the placing of the impaired contralateral forelimb when rats were able to use visceral and visual cues (tests 2 and 4) (Fig. 3B). In contrast to the impaired forelimb, hindlimb-placing recovered quickly to the level of sham-operated rats and was not affected by the drug treatment (Fig. 3C). In the foot-slip test, subacute treatment with atipamezole decreased the slip ratio (slips/taken steps × 100%) of impaired contralateral forelimb on days 3 and 7 after

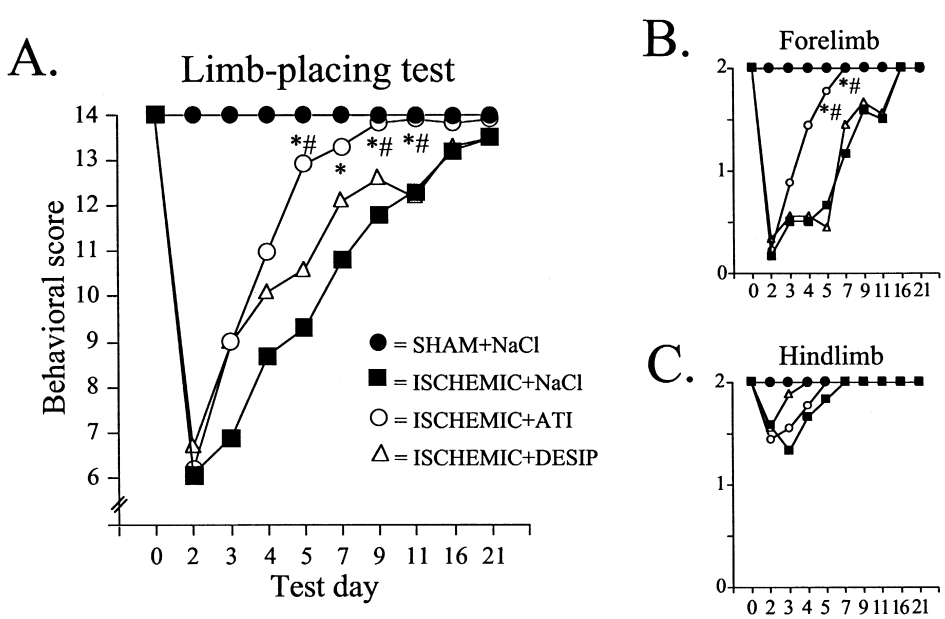

Fig. 3. Performance of rats in the limb-placing test after transient occlusion of the middle cerebral artery. Each value represents mean of 10–13 rats. (A) Total behavioral scores of limb-placing tests, (B) the scores for lateral placement of the affected forelimb (test 4), and (C) the scores for lateral placement of the affected hindlimb (test 5). Statistical significance: $^{*}P < 0.05$ (compared to ischemic saline controls); $^{\#}P < 0.05$ (compared to ischemic desipramine-treated rats).

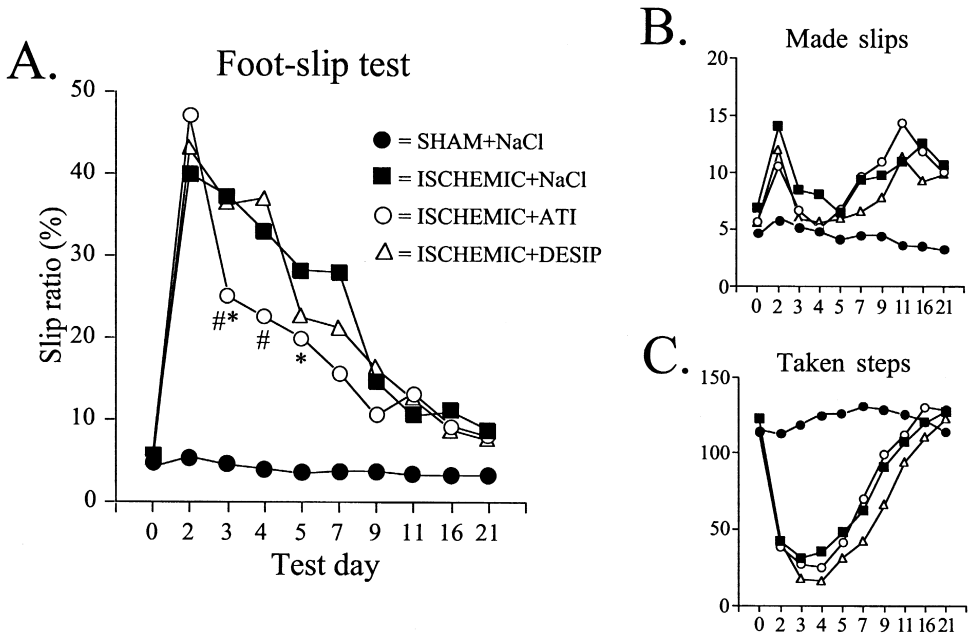

Fig. 4. Performance of rats in the foot-slip test after transient occlusion of the middle cerebral artery. Each value represents mean of 10–13 rats. (A) Slip ratio (slips/taken steps during 2-min recording), (B) taken steps (during the recording) and (C) made slips (during the recording). Statistical significance: $^{*}P < 0.05$ (compared to ischemic saline controls); $^{\#}P < 0.05$ (compared to ischemic desipramine-treated rats).

ischemia induction (Fig. 4A). The drug effect was not observed, however, when slips and steps where analyzed separately (Fig. 4B and C). The subacute treatment of atipamezole did not affect performance in the beam-walking test. The escape latency of ischemic rats to find the hidden platform was longer compared to sham-operated saline controls and the water-maze deficit was not alleviated by subchronic atipamezole treatment. There were no differences between experimental groups in swimming speed.

3.3. Effects of a single administration of atipamezole

A single administration of atipamezole (1 mg/kg) on day 2 after ischemia induction did not affect the spontaneous recovery rate of ischemic rats in the sensorimotor tests or water-maze test.

3.4. Effects of desipramine treatment

Acute or subchronic desipramine treatment did not affect performance of ischemic rats in any of the behavioral tests.

4. Discussion

The present study demonstrated improved behavioral performance of ischemic rats following subacute treatment with the selective α_2-adrenoceptor antagonist, atipamezole. The facilitatory effect was observed in behavioral tests assessing sensorimotor functions, but not in the Morris water-maze test. A single administration of atipamezole or a blockade of noradrenaline reuptake by desipramine had no significant effect on the behavioral outcome of ischemic rats.

Rats subjected to a transient occlusion of the middle cerebral artery were initially severely impaired in behavioral tests but exhibited a spontaneous recovery within 2 to 3 weeks almost to the level of sham-operated rats. A similar recovery profile has been previously noted in many simple behavioral tasks following cerebral ischemia whereas the deficit in cognitive tasks (e.g., Morris water-maze) or complex skilled motor tasks (e.g., Montoya's staircase test) is more long-lasting or even permanent (Markgraf et al., 1992; Grabowski et al., 1993). The atipamezole dose used in the present study (1 mg/kg) blocked central α_2-adrenoceptors (Haapalinna et al., 1997) and increased noradrenaline release (Laitinen et al., 1995) with no apparent side effects such as ipsiversive circling of ischemic rats, which is typical even with a low doses of amphetamine following striatal damage. The drug treatment was started on the second day after ischemia induction. This delay was selected to affect only the recovery process and to avoid any interference with ischemic neuronal damage. Thus, there were no differences in the infarct volumes between experimental groups. Two days after ischemia induction the rats were moved to an enriched environment and tested 30 min after drug administration. The rationale for transferring rats to the enriched

environment is the suggestion that amphetamine improves hindlimb locomotor ability and forelimb-placing following sensorimotor lesions, possibly through the noradrenergic system, when combined with task-relevant practice during the period of drug action (Feeney et al., 1982; Schmanke and Barth, 1997). It is also possible that the intensive behavioral testing and housing in an enriched environment has harmful effects. Recent reports indicate that early training or overuse of impaired forelimbs causes expansion of the lesion and hinders behavioral recovery following brain injury (Humm et al., 1998; Risedal et al., 1999). This is unlikely to be case in the present study where massed or forced use of the impaired limbs was avoided.

Subacute administration of atipamezole improved performance of ischemic rats in the limb-placing test and to some extent in the foot-slip test, but not in the beam-walking test. Forelimb-placing, when rats were able to use visceral and visual cues was particularly enhanced by the drug treatment. Hindlimb-placing was only slightly impaired and was not affected by atipamezole treatment, possibly because the hindlimb representation area in the cortex is partially spared in the transient ischemia model used in the present study. Given the quick recovery of hindlimb-placing, the slow recovery in the beam-walking test, which assesses mainly hindlimb function, was somewhat surprising. The slow recovery rate might be explained by fear or a lack of motivation in the ischemic rats, due to the spreading of the ischemic damage to brain areas such as the amygdala. Alternatively, the large striatal lesions might have caused difficulties in adjusting posture and/or initiating movements, therefore, delaying the maximal performance in the beam-walking test (Schmanke et al., 1996). The finding that atipamezole did not improve performance in the beam-walking test was also unexpected, but noradrenergic stimulation by atipamezole might in fact worsen the symptoms of ischemic rats with striatal damage, which in turn could counteract the overall beneficial drug effects (Mintz and Tomer, 1986). The foot-slip data on accurate placing of impaired forelimbs on the rungs of the motorized wheel were consistent with the limb-placing test. When steps and slips were analyzed separately, there were no significant differences between experimental groups. Possibly, the decrease in the number of steps taken and the parallel increase in the number of slips following ischemia induction contributed to the significant drug effect on the slip ratio. Consistent with previous findings (Haapalinna et al., 1997), the number of steps taken during the 2-min monitoring period was not different in drug-treated rats, indicating that atipamezole does not increase overall motor activity. In contrast to subchronic administration, a single dose of atipamezole did not affect the behavioral performance of ischemic rats. A single administration of amphetamine has an enduring recovery-facilitating effect in rats with sensorimotor cortex lesions (Feeney et al., 1982). The longer half-life of amphetamine or its non-specific effects and the different experimental model, however, might account for the different results between the present study and that of Feeney et al. (1982).

A subacute or single administration of the noradrenaline reuptake blocker, desipramine, did not affect behavior of ischemic rats in sensorimotor tests. Given the increase of synaptic noradrenaline following desipramine, we expected to find a behavioral improvement similar to that following atipamezole. The present results might be due to the different action of the drugs. Atipamezole stimulates the release of noradrenaline, possibly overwhelming the uptake capacity, whereas desipramine acts more passively by blocking the reuptake of already released amines. In addition, because of the long half-life of desipramine (10 to 35 h), chronic treatment is likely to result in the downregulation of α- and β-adrenoceptors (Barturen and Garcia-Sevilla, 1992). Adrenoceptors are important in functional recovery of rats following cortical trauma; blockade of β-adrenoceptors by propranolol does not affect recovery whereas blockade of α_1-adrenoceptors by prazosin retards recovery (Feeney and Westerberg, 1990). In addition, behavioral deficits of recovered rats could be reinstated by prazosin, indicating that α_1-adrenoceptors also have an important role in maintaining the recovered state (Sutton and Feeney, 1992). The deficit in limb-placing or beam-walking of recovered ischemic rats could not be reinstated by prazosin (unpublished data). A single injection of desipramine, which is unlikely to induce receptor downregulation, did not have an effect on the behavior of the ischemic rats. A single administration of desipramine (6 or 19 mg/kg) facilitates motor performance following unilateral lesions of the sensorimotor cortex (Boyeson and Harmon, 1993). Taken together, the mechanisms underlying promotion of recovery as well as maintenance of the recovered state might be different following cortical lesions and a more widespread damage caused by transient occlusion of the middle cerebral artery.

Ischemic rats had significantly longer escape latencies and path lengths in the Morris water-maze compared to sham-operated rats. This is consistent with previous studies indicating that there is a spatial memory disturbance after focal cerebral ischemia in rats (Yonemori et al., 1996, 1999). Neither acute nor subacute treatment with atipamezole or desipramine alleviated the water-maze deficit. Acute treatment with atipamezole either before or after tasks assessing learning and memory improved the performance of rats (Haapalinna et al., 1998). The present water-maze test was conducted almost 2 weeks after cessation of the treatment, which might partially account for the results. This indicates, however, that drug-treatment did not result in permanent improvement in water-maze performance.

The time course of improved behavioral performance of ischemic rats following atipamezole treatment might be related to mechanisms underlying the recovery process. The early behavioral deficit might be partially due to the overall condition of the rats after operation. Usually, weight

gain normalized within a few days after ischemia induction and the rats were able to take care of themselves. Severe edema after a large hemispheric lesion also contributes to the initial behavioral deficit (Dombovy and Bach-y-Rita, 1988). Whether atipamezole enhances resolution of edema cannot be excluded, but the positive drug effect observed at later phases of treatment does not support this idea. Furthermore, the resolution of diaschisis, i.e., functional depression of remote brain areas such as the contralateral cerebellum, is suggested to contribute to the behavioral deficits following brain injury (Feeney and Baron, 1986). Damage to noradrenergic terminals in the cortex produces retrograde inhibition of locus coeruleus activity (Feeney et al., 1982), which is reflected as a decrease in the noradrenaline content and a decreased release in the contralateral cerebellum (Krobert et al., 1994). Amphetamine transiently and partially restores noradrenaline release in the cerebellum (Krobert et al., 1994), which in turn might contribute to behavioral improvement. The atipamezole dose used in the present study (1 mg/kg, s.c.) increases noradrenaline release in the hypothalamus during its half-life (Laitinen et al., 1995) and it remains to be seen whether this is also the case in the cerebellum. The fact that the rats did not show improvement in limb-placing or beam-walking tests 0 to 90 min after the first drug administration or after the following two or three drug administrations, suggests that repeated dosing combined with task-relevant experience might be needed to achieve recovery. In addition, the significant difference between ischemic saline controls and atipamezole-treated rats from day 5 onwards after ischemia induction suggests plastic reorganization that contributes to behavioral improvement. Increased growth-associated protein (GAP-43) (days 3 to 14) and synaptophysin (days 14 to 60) immunostaining were recently reported following amphetamine administration to rats subjected to permanent occlusion of the middle cerebral artery (Stroemer et al., 1998). The improved performance of ischemic rats in the present study temporally correlated with the previously demonstrated increase in GAP-43 staining in cortical areas adjacent to the lesion.

In conclusion, although the exact mechanisms and anatomic substrates underlying improved behavioral performance of ischemic rats following α_2-adrenoceptor blockade by atipamezole are not known, the present results further suggest that repeated use-dependent release of noradrenaline has an important role in the motor recovery process.

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