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Involvement of α_2-Receptors in the Analgesia Induced by Transient Forebrain Ischemia in Rats

EMILIO MERLO PICH,*†[1] ROBERTA GRIMALDI,* ISABELLA ZINI,* ANDREA FRASOLDATI,*† PAOLO MARRAMA† AND LUIGI F. AGNATI*[2]

**Institute of Human Physiology and †Endocrinology, University of Modena, via Campi 287, 41100 Modena, Italy*

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MERLO PICH, E., R. GRIMALDI, I. ZINI, A. FRASOLDATI, P. MARRAMA AND L. F. AGNATI. *Involvement of α_2-receptors in the analgesia induced by transient forebrain ischemia in rats.* PHARMACOL BIOCHEM BEHAV **45**(3) 607–614, 1993.—Transient forebrain ischemia induced in rats by the four-vessel occlusion method produced analgesic effects in the hotplate test that persisted for 2 weeks. Ischemia-induced analgesia was attenuated by low doses of α_2-agonist clonidine (0.01–0.10 mg/kg, IP) and enhanced by low doses of α_2-antagonists yohimbine (1–2 mg/kg, IP) and idazoxan (0.25–1.00 mg/kg, IP) administration 7 days after ischemia. Ischemia-induced analgesia was not affected by methysergide, naloxone, propranolol, or phenoxybenzamine administered 7 days after ischemia, when motor control and arousal level of rats recovered to normal conditions. The enhanced response to yohimbine was antagonized by pretreatment with clonidine (0.75 mg/kg, IP) and naloxone (10 mg/kg, IP), suggesting the involvement of endogenous opioid peptides. The enhanced response to yohimbine was still present 2 months after ischemia, when preischemic hotplate threshold was restored. As α_2-agonists reduce and α_2-antagonists increase the outflow of central noradrenaline, it is suggested that activation of central noradrenergic systems is involved in the mediation of ischemia-induced analgesia.

Cerebral ischemia Four-vessel occlusion model Pain Rat Hotplate test Tail-flick test Yohimbine Idazoxan Clonidine Naloxone

A series of neurological and behavioral impairments have been associated with brain injury that follows spontaneous or experimental cerebral ischemia (4,34,36,42). In 1979, Pulsinelli and Brierley proposed a procedure of transient, global forebrain ischemia in the rat as a model of cardiac arrest, the four-vessel occlusion method. Reproducible neuronal damage in various telediencephalic areas was obtained with this method (31,32). Twenty to 30 min of transient forebrain ischemia result in impairments of arousal (31,44), motor control (4), locomotor activity (24), passive avoidance (45), and T-maze and radial maze learning (6,42,43). Some studies in four-vessel-occluded rats showed that most of the behavioral impairments affecting arousal and motor activity subsided within a 1–2 days [(4); Merlo Pich, unpublished observation], whereas learning deficits persisted for longer periods (6,42, 43). However, to our knowledge no study has investigated the effects of transient forebrain ischemia on pain threshold.

Our interest in this problem stemmed from the observation that most of the brain areas injured by transient forebrain ischemia, that is, the somatosensory cortex, hippocampus, and thalamus, are involved in the processing of pain-related signals. In addition, biochemical evidence has shown that cerebral ischemia largely affects central monoamine- and peptide-containing systems (3,14,16,18,21,30), whose interplay has been known to modulate the threshold of pain in mammals (1,10). Finally, analgesia has been reported after exposure to various stressors (1), and transient cerebral ischemia is a powerful stressor.

The purpose of the present investigation was two-fold: a) to study the time course of pain threshold changes following four-vessel occlusion using two different tests, namely, the tail-flick test and the hotplate test; and b) to study the pharmacological susceptibility of pain threshold to drugs interfering with noradrenaline, serotonin, and opioid peptide transmission administered at various time intervals after transient forebrain ischemia.

[1] Current address: Department of Neuropharmacology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037.
[2] To whom requests for reprints should be addressed.

METHOD

Animals

Adult, male Sprague-Dawley rats (Charles River, Italy) weighing 250–275 g were kept in a controlled environment on a 12 L : 12 D schedule (light 0800–2000 h). Food and water were provided ad lib. After transient forebrain ischemia or sham procedure, rats were housed individually.

Surgical Procedure

Transient forebrain ischemia was induced as described by Pulsinelli and Brierley (1979) with modifications (44). Rats were anesthetized with ketamine HCl (Ketalar-Parke Davis) (100 mg/kg, IP). Both common carotid arteries were exposed through a ventral midline cervical incision and isolated from the accompanying nerves and veins. A nontraumatic silk thread (EP15ex4/0) was loosely placed around each common carotid artery without interrupting carotid blood flow and the incision was closed with a single suture. A second dorsal midline incision from the occipital protuberance to the medial cervical region was made. The paraspinal musculature was separated from the midline and, with the use of an operating microscope, the right and left alar foramina of the first cervical vertebrae, beneath which the vertebral arteries pass traveling within the vertebral canal, were exposed. A 0.5-mm diameter electrocautery needle (Coagulasem 500 Bipolar Electrocautery) was inserted through each alar foramen and both vertebral arteries were electrocauterized and permanently occluded. The electrocautery was grounded through the animal's foreleg. While animals were still anesthetized, electrodes (stainless steel screws, Breitfeld and Schliewert, Bad Vilbel, Germany) were inserted into the skull bone overlying the frontoparietal cortex to perform electroencephalographic (EEG) recordings (Battaglia Rangoni poligraph, AFL channel, A380-AC series).

Rats were then allowed to recover from anesthesia for 24 h, when they appeared normal according to gross behavior assessment and EEG recordings. Awake rats were then hand held, the ventral neck suture gently removed, and both carotid arteries transiently occluded with stainless steel clips (Biemer-Clip 0.29–0.39, Aesculap-Werke, Tuttlingen). Body temperature was monitored with a rectal thermometer and animals were kept under a heating lamp until thermal homeostasis was restored. The carotid clips were removed 30 min later and restoration of blood flow through these arteries was verified by direct inspection. During the ischemic period, animals were observed for their level of arousal, the presence or absence of righting and corneal reflexes, their ability to walk and climb, and their EEG activity. Only those animals immediately losing their righting reflex, unresponsive to pain stimuli for 20–30 min after bilateral carotid occlusion, with EEG becoming isoelectric within 2–3 min after bilateral carotid occlusion and maintaining abnormal pattern throughout the occlusion period were included in the study. Finally, rats that exhibited seizures during recovery were excluded from the study.

Pain Tests

The nociceptive threshold was evaluated by using either the tail-flick method (5) or the hotplate test (9). The tail-flick test was performed by using the Socre Tail Flick Unit (Basile, Italy). Briefly, the rat tail is kept under a collided light beam producing a reliable thermal nociceptive stimulus. The ensuing lateral flicking of the tail interrupts a photocell beam connected to an electronic timer, giving the tail-flick latency measurement. Basal tail-flick pain threshold was determined by the mean tail-flick latencies from three measurements made 10 min apart.

The hotplate test was performed using a Hotplate Unit (Basile, Italy). The platform temperature was set to 54.50 ± 0.2°C. A Plexiglas cylinder 23 cm in diameter and 30 cm high was used to confine the rat on the hotplate. The latency to lick the hindpaw was recorded (to the nearest 0.1 s). In the absence of this behavior, the trial was terminated after 30 s. The basal pain threshold was determined by the mean of two measurements made 30 min apart. When drugs were administered, hotplate latency was measured −5, 30, 60, 90, and 120 min after drug injection. All the tests were performed by an experimenter unaware of the treatment and started between 1500 and 1700 h. After testing, rats were returned to their home cage and maintained in standardized conditions until the next experimental day.

Histology

At the end of the experiments, animals were anesthetized and sacrificed by intracardiac perfusion with 50 ml warm saline followed by 100 ml ice-cold 4% paraformaldahyde + 0.14% picric acid in phosphate buffer (0.1 M, pH 7.2). Each brain was dissected out, postfixed overnight, cut in a cryotome (40-μm thick sections) at two rostrocaudal levels (bregma: −0.70 and −2.80 mm) (29), and stained with cresyl violet for histological assessment. Lesions were evaluated using a neuropathologic score (6,31). According to these criteria, ischemic neuronal damage in the hippocampus and striatum was graded on a scale of 0–3. Only animals whose histological analysis revealed severe neural damage (i.e., neuropathological score of CA1–CA3 hippocampal field and in dorsolateral striatum higher than 2.5) were included in the study.

Drugs

Yohimbine, clonidine, idazoxan, (−)propranolol, naloxone (Sigma Chemical Co., St. Louis, MO), phenoxybenzamine (SK&F, Philadelphia, PA), and methysergide (Sandoz, Basel) were used. All drugs with the exception of yohimbine and naloxone were dissolved in 0.9% saline. Yohimbine was dissolved in distilled water and naloxone in saline 0.86% and methylhydroxybenzoate 0.18%. A volume of 1 ml/kg body weight of drug solution was used for each injection. All drugs were freshly dissolved before each experiment and injected IP.

Procedures

In the first experiment, pain threshold was studied in four-vessel-occluded rats (n = 8) and sham-operated rats (n = 8) by means of the tail-flick procedure 4 h and 1 day after ischemia. Hotplate latency was measured in four-vessel-occluded rats (n = 24) and sham-operated rats (n = 12) 4 h and 1, 2, 4, 6, 9, 14, 30, and 60 days after ischemia. On the testing day, rats were removed from their home cages and placed in the tail-flick apparatus or on the hotplate apparatus for basal pain threshold assessment. During intertest intervals, rats were kept in their home cages.

In the second experiment, the pharmacological susceptibilities of analgesia observed in rats 1 and 7 days after ischemia were tested by administering a number of drugs that antagonize central transmission of monoamines or opioid peptides. On day 1 after ischemia, 69 ischemic rats and 60 sham-operated rats were assigned to different treatment groups.

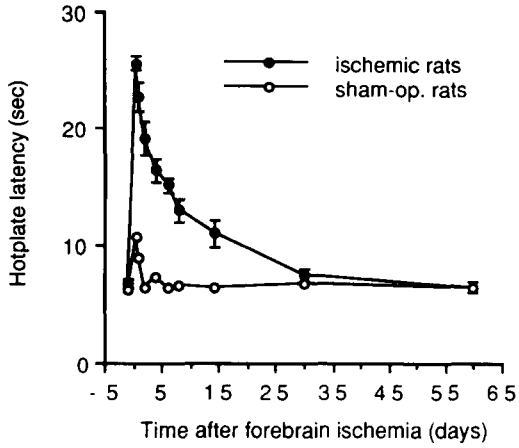

FIG. 1. Recovery curves of pain threshold in ischemic rats (n = 24) and sham-operated rats (n = 12). Pain threshold was measured using hotplate with a cut-off period of 30 s. Transient forebrain ischemia was obtained with the four-vessel occlusion procedure, with 30 min of carotid occlusion. Data are expressed as mean ± SEM. A posteriori multiple-comparison analysis showed significant ($p < 0.01$) differences between the two groups at all time intervals except 30 and 60 days. Exponential models were used to describe these recoveries (see text).

Each group consisted of five to seven rats. After basal hotplate latency measurement, the serotonin antagonist methysergide (2.5, 5.0, and 15.0 mg/kg), α_2-receptor antagonist yohimbine (0.2, 1.0, and 2.0 mg/kg), and naloxone, an opioid receptor blocker (1.2, 2.5, and 10.0 mg/kg), were administered. On day 7 after ischemia, the same rats tested on day 1, 47 naive ischemic rats, and 45 naive sham-operated rats were randomly assigned to different treatments, groups consisting of five to seven rats each. The following drugs were administered: naloxone (2.5 and 10.0 mg/kg), methysergide (2.5 and 15.0 mg/kg), yohimbine (0.2, 1.0, and 2.0 and 10.0 mg/kg), the selective α_2-receptor antagonist idazoxan (0.25 and 1.00 mg/kg) (8), the α_1-α_2-receptor antagonist phenoxybenzamine (2.5 and 15.0 mg/kg), and (−)propranolol (1.0 and 4.0 mg/kg), a β-receptor antagonist. The drugs were injected 5 min after measurements of basal hotplate latency. Each animal was used as its own control. Vehicle-injected animals were used in most of the experiments as controls for the dose-response analysis.

In a third group of experiments, the effects of the selective α_2-receptor agonist clonidine (0.01, 0.10, 0.75, and 1.00 mg/kg) on hotplate latency was studied in ischemic and sham-operated rats. All these tests were performed 7 days after ischemia, when all animals fully recovered from impairment of motor control and arousal levels observed in the early period (1-2 days) after ischemia (Merlo Pich, unpublished observations). In separate groups of ischemic rats (n = 5-7 per treatment), clonidine at a dose that does not affect basal hotplate latency (0.75 mg/kg) and two doses of naloxone (2.5, 10.0 mg/kg) and methysergide (2.5, 15.0 mg/kg) were administered 30 min before administration of 2.0 mg/kg yohimbine. This experiment was performed to demonstrate the α_2-receptor specificity of the yohimbine-dependent enhancement of ischemia-induced analgesia and the possible involvement of endogenous opioid peptide or serotonin systems.

Finally, 2 months after transient forebrain ischemia ischemic rats (n = 21) and sham-operated rats (n = 21) were administered yohimbine (0.2, 1.0, and 2.0 mg/kg) for hotplate testing. By this time, basal hotplate latency returned to preischemic values and did not differ from those of sham-operated rats. This experiment was designed to assess the presence of the yohimbine-dependent effects on hotplate latency in a period in which ischemic lesions were stabilized and functional recovery was almost complete.

Statistics

Linear and exponential regression analyses were used to fit the data of the pain threshold time course after ischemia or sham operation. The results from experiments on the effects of drug administration on the hotplate test in ischemic and sham-operated rats were analyzed by two-way analysis of variance (ANOVA) for repeated measurements (group × time in-

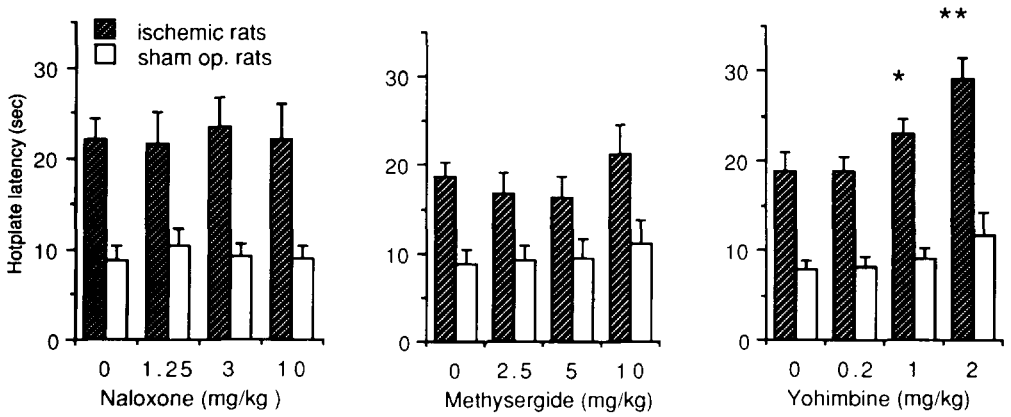

FIG. 2. Effects of naloxone, methysergide, and yohimbine (IP) on hotplate latency in ischemic and sham-operated rats 1 day after ischemia. Hotplate latency was measured −5, 30, 60, 90, and 120 min after drug injection. The values reported in the figure are those measured 30 min after drug injection. Vehicle treatment is represented as "0" dose. Data are expressed as mean ± SEM. Each treatment group consisted of five to seven rats (*$p < 0.01$, vs. vehicle-treated rats; Tukey test). Latencies in ischemic rats were always significantly ($p < 0.05$) higher than in sham-operated rats.

terval after drug administration). Two-way ANOVA was also used to analyze the effect of various doses of a drug on the pain threshold measurements in ischemic and sham-operated rats at a fixed time interval (group × dose treatment). These procedures were followed by a posteriori Tukey's or Newman-Keuls test for multiple comparisons. The same parametric tests were used for the analysis of data from the experiment on antagonism of the enhanced response induced by yohimbine.

RESULTS

Measurements of pain threshold in rats obtained 4 h and 1 day after carotid artery occlusion gave different results according to the procedure used. In ischemic rats, tail-flick latency measured before ischemia (4.92 ± 0.30 s) did not statistically differ from the results obtained 4 h and 1 day after ischemia (4.79 ± 0.28 s and 4.81 ± 0.27 s, respectively). In contrast, hotplate latency showed marked effects in ischemic rats at various time intervals postischemia (Fig. 1). The highest values were measured 4 h and 1 day after carotid occlusion, while progressively lower values were measured on days 2, 4, 7, 9, and 14, all the values being significantly different from corresponding sham-operated control measurements ($p < 0.01$). At 30 and 60 days, no difference between the two groups was noted. The best fit of the data from ischemic and sham-operated rats was obtained using exponential regression models. The fitting procedure resulted in the two following equations, which described the recovery curve of pain threshold after ischemia in the two groups:

$$Y = 3.119*e^{-0.210t} \text{ in sham-operated rats, } (r^2 = 0.69);$$
$$Y = 14.472*e^{-0.012t} + 15.256*e^{0.514t} \text{ in ischemic rats,}$$

where Y denotes the hotplate latency (seconds) and t the time after transient forebrain ischemia (day). A biexponential model produced the best fitting of the data from ischemic rats ($r^2 = 0.99$), suggesting the presence of one fast and one slow component. The fast component explained the changes in hotplate latency during the early period after ischemia (i.e., within the first 3–5 days), whereas the slow component accounted mainly for late effects (i.e., approximately between 6 and 60 days).

The pharmacological susceptibilities of analgesia induced by transient forebrain ischemia 1 day and 7 days after ischemia are shown in Figs. 2 and 3, respectively. In these figures, only the values of hotplate latency measured 30 min after drug administration are reported. On the first day after ischemia, the high baseline pain threshold was not significantly affected by either naloxone at the doses of 1.2, 2.5, and 10.0 mg/kg or methysergide at the doses of 2.5, 5.0, and 15.0 mg/kg. In contrast, 1.0 and 2.0 mg/kg yohimbine produced a significant increase in the hotplate latency in ischemic rats ($p < 0.01$), whereas no effect was observed in sham-operated rats. No significant increase of hotplate latency was found with 0.2 mg/kg yohimbine in either ischemic or sham-operated rats.

On the seventh day after ischemia, the basal hotplate latency was still significantly higher in ischemic rats than in sham-operated rats ($p < 0.01$). No effect was observed in ischemic rats with naloxone, methysergide, (−)propranolol, and phenoxybenzamine (Fig. 3), whereas yohimbine (1.0 and 2.0 mg/kg) and idazoxan (0.25 and 1.00 mg/kg) increased the hotplate latency in a dose-dependent fashion (Fig. 4). In sham-operated rats, yohimbine did not affect hotplate latency at any dose. In ischemic rats, 0.2 mg/kg yohimbine was without effect, whereas 5.0 and 10.0 mg/kg yohimbine produced hypotonia and sedation, preventing further testing. A similar effect was also obtained with 15.0 mg/kg phenoxybenzamine. The time course of acute effects of various doses of yohimbine and idazoxan in ischemic and sham-operated rats is showed in Fig. 4. Two-way ANOVA for repeated measurements revealed significant group ($p < 0.01$) and time ($p < 0.01$) effects for 1.0 and 2.0 mg/kg yohimbine and for all idazoxan doses, respectively.

The effects of clonidine on hotplate latency 7 days after ischemia are shown in Fig. 5. Low doses of clonidine (0.01 and 0.10 mg/kg) produced a significant decrease of hotplate latency in ischemic rats ($p < 0.05$) but not in sham-operated rats 30 min after drug administration. Conversely, 1.00 mg/kg clonidine produced a significant analgesic response in both ischemic and sham-operated rats. At this dose, no significant differences were found between the two groups. No effects were observed in either ischemic or sham-operated rats when clonidine was administered at the dose of 0.75 mg. This dose was used to study the antagonism of the enhanced analgesic

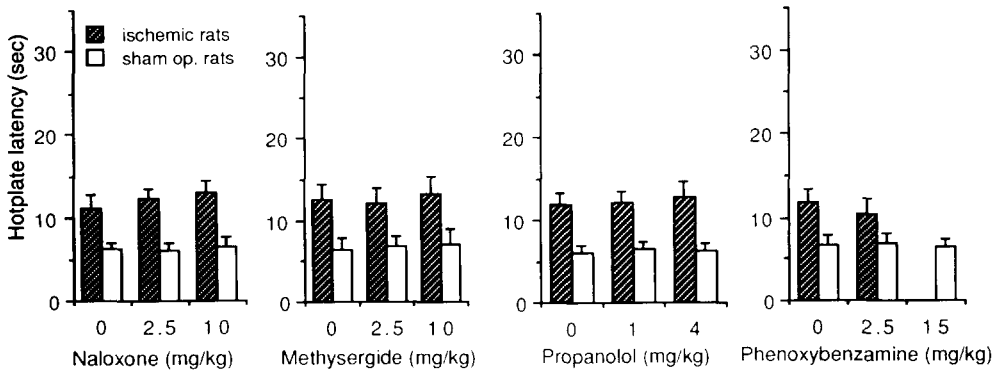

FIG. 3. Effects of naloxone, methysergide, propranolol, and phenoxybenzamine (IP) on hotplate latency in ischemic and sham-operated rats 7 day after ischemia. Hotplate latency was measured −5, 30, 60, 90, and 120 min after drug injection. The values reported in the figure are those measured 30 min after drug injection. Vehicle treatment is represented as "0" dose. Data are expressed as mean ± SEM. Each treatment group consisted of five to seven rats (*$p < 0.01$, vs. vehicle-treated rats; Tukey test). Latencies in ischemic rats were always significantly ($p < 0.05$) higher than in sham-operated rats.

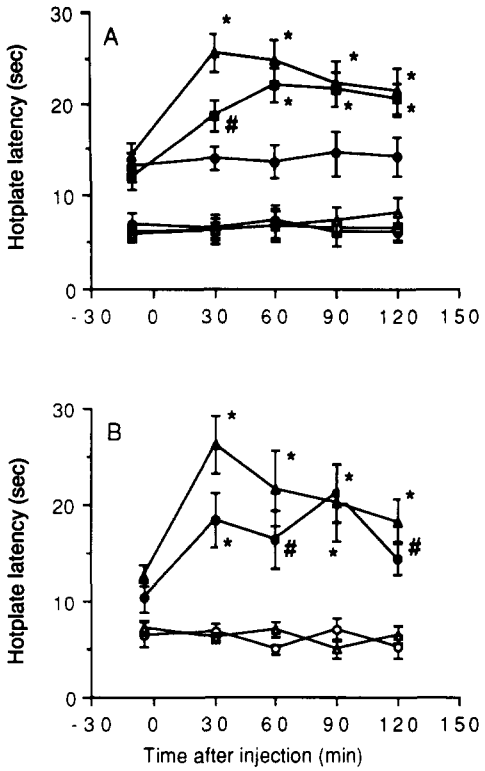

FIG. 4. Effects of various doses of yohimbine (A) and idazoxan (B) on hotplate latency in ischemic and sham-operated rats 7 days after ischemia. The data are shown as mean ± SEM. Each treatment group consisted of five to eight rats. In both panels, the closed symbols correspond to values obtained in ischemic rats and the open symbols correspond to values obtained in sham-operated rats. Doses of yohimbine (IP): 0.2, 1.0, and 2.0 mg/kg. Doses of idazoxan (IP): 0.25 and 1.00 mg/kg (#$p < 0.05$, *$p < 0.01$, vs. time "0" level, Tukey test).

response induced by yohimbine in ischemic rats. Clonidine (0.75 mg/kg, IP) completely antagonized the effect of 2.0 mg/kg IP yohimbine on the hotplate test (Fig. 6). Treatments with 2.5 or 15.0 mg/kg methysergide did not significantly change the enhanced response induced by 2.0 mg/kg yohimbine in ischemic rats, while significant attenuations were observed after 2.5 and 10.0 mg/kg naloxone ($p < 0.05$ and $p < 0.01$, respectively; Fig. 5).

The effects of yohimbine administration in ischemic and sham-operated rats 60 days after ischemia are shown in Table 1. In ischemic rats, 1.0 and 2.0 mg/kg yohimbine produced significant increases of hotplate latency at various time points after administration ($p < 0.01$); these values also differed significantly from those of sham-operated rats ($p < 0.01$).

DISCUSSION

Thirty minutes of transient forebrain ischemia produced a temporary increase of hotplate latencies in rats that persists for 2 weeks. In contrast, no effect was found by testing ischemic rats in the tail-flick procedure 4 h and 1 day after ischemia, when the hotplate latency measurements were maximal. During the early period after ischemia (1–2 days), impaired motor control and arousal level were observed in rats, suggesting that nonspecific factors participate in increasing hotplate latencies. Hotplate latencies were still elevated 4 days after ischemia, when the gross behavior of animals was normal. On days 1 and 7 after ischemia, hotplate response was not affected by naloxone and methysergide, drugs known to interfere with endogenous opioid peptides and serotonin neurotransmission, whereas the α_2-antagonists yohimbine and idaxozan significantly increased hotplate latencies of ischemic rats in a dose-related fashion. Seven days after ischemia, attenuation of the ischemia-induced analgesia was observed after low doses of the selective α_2-agonist clonidine. In addition, clonidine antagonized the yohimbine-induced enhancement of hotplate latency. These results suggested that central α_2-receptors were involved in mediating both ischemia-induced analgesia and the enhancing effect of yohimbine on hotplate latency in ischemic rats. The latter effect was also antagonized by naloxone, indicating the mediation of endogenous opioid peptides. Finally, the effect of yohimbine on hotplate latency was found 60 days after the ischemic insult, when the basal hotplate latency had recovered to preischemic values. The prolonged elevation of hotplate latencies observed after brain ischemia differs from the temporal profile described for stress-induced analgesia (1), suggesting different underlying mechanisms. In general, the analgesic effects of exposure to a stressor last between a few hours and 2 days (1). As surgery is a powerful stressor in rodents (13), a further demonstration of the short-lasting analgesia induced by stress is provided by sham-operated rats of the present experiment.

Dissociation between responses obtained in the tail-flick test and hotplate test have been reported in the literature (7,39). In the tail-flick test, the response is principally governed by a neuronal loop at the spinal level (1,5), whereas the hotplate test has been thought to involve an integrated escape response that may be mediated at CNS levels higher than the spinal cord (1,9). If this interpretation is correct, the observed dissociation between tail-flick and hotplate results suggests that transient forebrain ischemia may affect the integrated escape response assessed in hotplate testing by impairing brain circuits located above the spinal cord.

The increase of hotplate latency induced by transient forebrain ischemia appeared most intense on the 1st, 2nd, and 4th days, decreasing until the 30th day, when the pain threshold recovered to preischemic values. These data were interpolated

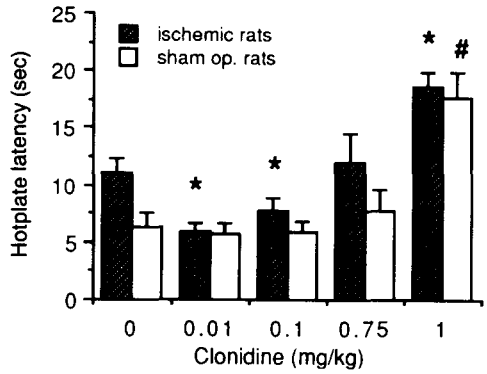

FIG. 5. Effect of various doses of clonidine on hotplate latency in ischemic and sham-operated rats 30 min after drug administration. Hotplate tests were performed 7 days after ischemia. Data are expressed as mean ± SEM. Each group consisted of six to eight rats (*$p < 0.01$, vs. respective basal levels, Tukey test).

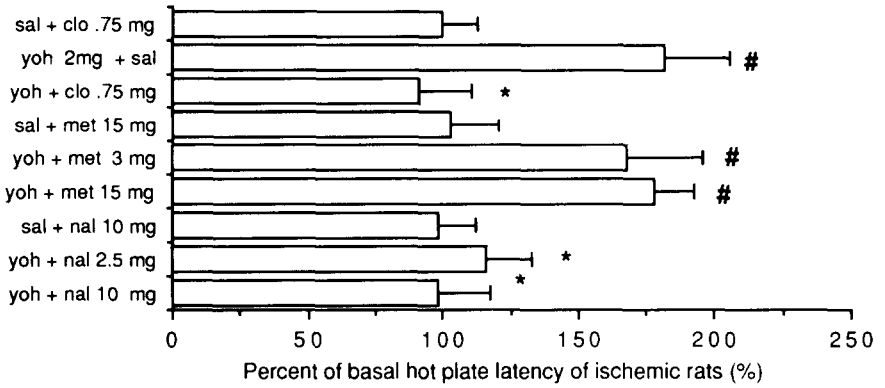

FIG. 6. Effects of clonidine (clo), methysergide (met), and naloxone (nal) on the enhancement of analgesic response induced by 2.0 mg/kg IP yohimbine (yoh) in ischemic rats. All tests were performed 7 days after ischemia. Hotplate latency was measured −5, 30, 60, 90, and 120 min after drug injection. The values reported in the figure are those measured 30 min after drug injection. Data are expressed as percent of mean values of the respective basal values ± SEM. Each group consisted of six to eight rats (#$p < 0.01$ vs. respective vehicle-treated rats, *$p < 0.05$ vs. respective yohimbine-treated rats; Tukey test).

using various regression models to obtain the recovery curves of the analgesic response in ischemic rats (20). The best fit was reached by using a biexponential regression model. A tentative interpretation suggested that the short half-time component of the equation might account for the recovery from acute impairment of motor control and arousal level, generally present 1–2 days after ischemia [(4); Merlo Pich, unpublished observations]. In the early period after ischemia, the integrated response measured in the hotplate test might be disrupted by severely impaired motor control and arousal level. The long half-time component of the equation might be interpreted as the result of more specific adaptive and restorative neural processes that underlie the recovery of hotplate latency to preischemic values. By day 4 after ischemia, motor impairments subsided, whereas hotplate latencies were still abnormally elevated. The slow decrease of hotplate latencies observed with time suggests that an adaptive reorganization of neural systems involved in pain control is in progress.

TABLE 1

EFFECTS OF VARIOUS NEUROTRANSMITTER ANTAGONISTS ON ISCHEMIA-INDUCED ANALGESIA IN THE HOTPLATE TEST MEASURED 30 min AFTER ANTAGONIST ADMINISTRATION AT 7 DAYS AFTER TRANSIENT FOREBRAIN ISCHEMIA IN RATS

Drugs and Doses	Hotplate Latency (seconds)	
	Ischemic Rats	Sham-Operated Rats
Methysergide (mg/kg, IP)		
0	13.6 ± 2.9	6.7 ± 2.5
2.5	13.4 ± 2.8	7.0 ± 1.2
10.0	14.2 ± 3.2	6.9 ± 2.5
(−)Propranolol (mg/kg, IP)		
0	12.8 ± 1.7	6.2 ± 1.5
1	12.9 ± 1.9	6.5 ± 1.1
4	13.8 ± 2.1	6.0 ± 1.0
Naloxone (mg/kg, IP)		
0	11.8 ± 2.4	6.6 ± 1.3
2.5	12.3 ± 1.6	6.4 ± 1.2
10.0	13.1 ± 2.1	7.0 ± 1.4
Phenoxybenzamine (mg/kg, IP)		
0	12.2 ± 2.6	7.2 ± 2.0
2.5	10.3 ± 2.1	7.3 ± 1.5
15.0	–	6.8 ± 1.8

In all groups, basal hotplate latencies did not significantly differ from those measured 30 min after drug administration. Data are expressed as mean ± SEM. Each treatment group consisted of five to seven rats.

Pain suppression has been described as a relevant mechanism of adaptation to changing environmental circumstances and proposed as a by-product of the activation of a constellation of systems that control the organism's response to stressful or damaging stimuli (1). The possible mediation of ischemia-induced analgesia by neural systems participating in pain control, such as noradrenaline-, serotonin-, and opioid peptide-containing systems, was assessed by administering agonist and antagonist drugs 7–8 days after ischemia, when the gross behavior of animals was fully recovered from postischemic impairments. Several doses of serotonin and opioid peptide antagonists did not affect ischemia-induced analgesia, whereas significant effects were obtained with clonidine, yohimbine, and idazoxan, suggesting a possible implication of noradrenaline-containing neurons in ischemia-induced analgesia. In vivo and in vitro studies indicate that α_2-agonists decrease and α_2-antagonists increase the release of noradrenaline when administered at low doses (2,22,23). These effects can be mediated by α_2-receptors located in the cell body of noradrenaline-containing neurons or presynaptically on the noradrenaline-containing nerve terminal. In our experiment, doses of clonidine known to reduce noradrenaline turnover in terminal brain areas (2) attenuated the ischemia-induced analgesia, whereas doses of yohimbine that increase central noradrenaline outflow were found to enhance the antinociceptive response of ischemia rats. The increased activity of nor-

adrenaline-containing neurons has been related to analgesia induction. Electrical stimulation of noradrenaline-containing neurons, in particular those located in the locus coeruleus, produces antinociceptive responses in rodents (37), and similar results are reported after low doses of yohimbine (7).

A plausible hypothesis concerning the modulatory effect of α_2-adrenergic drugs on ischemia-induced analgesia describes a mediation via noradrenaline-containing neurons whose activity has been increased in response to transient forebrain ischemia. Transitory activation of central noradrenaline-containing neurons in response to hypoxic-ischemic damage (3,19,14) or stressful stimuli (11,35,41) has been reported in rodents. Exposure to stressful stimuli induced a transient analgesia in mammals (1,26). In one experiment, low doses of clonidine antagonized the stress-induced analgesia in rats exposed to short-duration foot-shock (38). These results are similar to those observed in ischemic rats administered low doses of clonidine. In addition, administration of α-receptor antagonists resulted in enhancement of analgesia, suggesting the involvement of presynaptic mechanisms in noradrenaline-containing neurons. From this evidence, we suggested that the enhanced antinociceptive response induced by yohimbine in ischemic rats was mediated by an increase of outflow in noradrenergic terminal areas involved in pain control. Interestingly, in the present experiment the yohimbine-dependent enhancing effect observed in ischemic rats was antagonized by naloxone. Some studies indicate that naloxone attenuates the antinociceptive response induced by ICV administration of high doses of α-adrenergic agonists in the rat (25) and by prolonged exposure to intense stressors (1). In addition, naloxone decreases the elevated pain threshold of spontaneously hypertensive rats, a strain characterized by increased turnover of brain noradrenaline (40).

At the tested doses, we were not able to antagonize the ischemia-induced analgesia with postsynaptic α- or β-receptor antagonists. In particular, high doses of yohimbine and phenoxybenzamine produced severe hypotonia and sedation in ischemic rats but not in sham-operated rats. As the injections were performed intraperitoneally, possible peripheral effects on the cardiovascular system could not be ruled out (17). However, there is evidence that a certain level of activation of noradrenaline-containing neurons is necessary for arousal level maintenance and that enhanced activity of noradrenaline-containing neurons is reported after intervening environmental or physical challenges (12). It is reasonable to assume that in ischemic rats noradrenaline-containing neurons are highly activated to maintain arousal and basic autonomic functions despite the impairment induced by transient forebrain ischemia. Therefore, the blockade of noradrenergic transmission induced by massive doses of α-antagonists severely reduced the arousal of ischemic rats, preventing any behavioral assessment.

In our experiment, low doses of clonidine (0.01–0.10 mg/kg) reduced ischemia-induced analgesia but were ineffective in sham-operated rats, whereas high-dose clonidine (1 mg/kg) produced a strong analgesia in either ischemic or sham-operated rats. The analgesic effect of high-dose clonidine has been reported in one study where the dependent measure was the frequency of jumping behavior induced by intense foot-shock (28). It has been suggested that the antinociceptive action of high-dose clonidine is mediated by postsynaptic α_1-receptors at the spinal level (27,28,33,39), ruling out the involvement of α_2-receptors.

Finally, the enhanced analgesic response to yohimbine in the hotplate test 60 days after ischemia, when the reorganization of neural tissue after ischemic damage is almost complete (15), suggests the permanence of increased activity of noradrenaline-containing neurons. It is therefore reasonable to suggest that central noradrenaline systems are involved in restorative and adaptive processes that follow cerebral ischemic insult. From this point of view, measurements of hotplate latencies may give a functional estimate of postischemic recovery, suggesting a possible role for central noradrenaline systems in neural restorative processes.

In conclusion, the results of a series of pharmacological experiments indicate that the increases in hotplate latency found in rats after transient forebrain ischemia can be related to the increased activity of central noradrenaline-containing neurons controlling sensorimotor integration of pain stimuli. Speculatively, the increased activity of noradrenaline-containing neurons may serve to sustain arousal levels and promote postischemic restorative and adaptive processes. Ischemia-induced analgesia does not seem to be mediated by the opioid peptide system, but the analgesic response enhancement induced by yohimbine clearly showed a naloxone dependency. With time, the ischemia-induced analgesia attenuated and disappeared, leaving central noradrenaline-containing systems more sensitive to α_2-receptor blockade, as shown by the increase of hotplate latency induced by low-dose yohimbine in ischemic rats 60 days after transient forebrain ischemia.

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