Effect of the (3-4) Fragment of Substance P on Brain Ischemia in Rats with Different Types of Behavior

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A single administration of a fragment of substance P 30 min after ligation of the common carotid arteries increases resistance to brain ischemia primarily in rats with a passive type of behavior (improves their neurological status and prevents posthypoxic hyperactivity), prevents posthypoxic hyperactivity in rats with an intermediate type of behavior, and lowers the resistance of rats with an active type of behavior (increases their mortality and aggravates their neurological status).

Key Words: *brain ischemia; behavioral responses; substance P fragment*

Previously, we reported that the neuropeptide substance P(1-11) (SP) modulates the resistance of rats to circulatory hypoxia (ischemia) of the brain (CHB), the effect being dependent on the type of behavior [7]. For example, a single administration of SP 30 min after ligation of the common carotid arteries increases the resistance to CHB of rats with a passive type of behavior, decreases it in rats with an active type, and has no appreciable effect in rats with an intermediate type. The ability to induce different and often opposite responses is typical of peptides, including SP [1]. It has been hypothesized that the diversity of the effects of SP is associated with the cleavage of this substance in the organism by specific enzymes, since the N- and C-terminal fragments of SP produce opposite effects [13]. In addition, the properties of SP fragments differ depending on the length of the fragment and its amino acid composition. Generally, the N-terminal fragment of SP displays the same activity as the entire peptide, but, unlike the latter, has virtually no side effects [10,12]. In order to evaluate the antihypoxic activity of individual fragments of SP, we examined the effect of SP(3-4) on mortality, neurological status, and changes in the behavior of rats with different types of behavior during one month after the onset of CHB.

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MATERIALS AND METHODS

Experiments were performed on 116 outbred male rats weighing 250-300 g. The type of behavior was determined in the open field and forced swimming tests, as described elsewhere [4,6]. These tests make it possible to identify animals that significantly differ in three major behavioral parameters: the number of squares crossed, the number of upright postures (rearings), and the time of passive floating. Circulatory hypoxia of the brain was produced by bilateral ligation of the common carotid arteries [4,7]. Mortality, neurological status, and behavioral changes occurring after CHB were studied in 51 rats without administration of SP(3-4) and in 65 rats with its administration. The peptide was synthesized at the Institute of Molecular Pharmacology (Berlin) and kindly provided by Prof. P. Oehme. SP(3-4) was injected once intraperitoneally in a dose equimolar to 250 μg/kg SP 30 min after ligation of the carotid arteries. Previously it was demonstrated that this dose of SP increases resistance to emotional stress [8,10] and CHB [7]; SP(3-4) has a posi-

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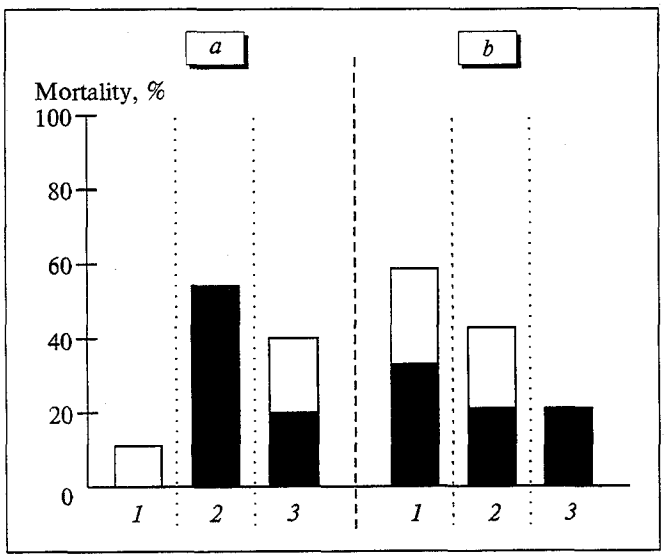

Fig. 1. Mortality in different groups of rats after the onset of brain ischemia without (*a*) and with (*b*) administration of SP(3–4). Black bars: mortality within a 48-h period; white bars: mortality within 1 month. Here and in Figs. 2 and 3: *1*) rats with an active type of behavior (9 and 12 animals); *2*) rats with an intermediate type of behavior (11 and 14 animals); *3*) rats with a passive type of behavior (10 and 14 animals).

tive effect on hypoxia caused by prenatal alcohol intoxication [2]. The condition of animals after ligation of the carotid arteries was evaluated on a 25-point scoring scale of neurological deficit (Table 1), which is our modification of the 100-point scale previously proposed for evaluation of neurological deficit in rats that have suffered clinical death [3]. The neurological status was the sum of points for all parameters. Behavioral changes after the onset of ischemia were determined in the open field test (on days 2, 7, and 30 after the onset of CHB) and the forced swimming test (on day 30) [11]. The results were processed using standard STATGRAPHICS software. The significance of differences in behavioral responses during different periods of CHB was estimated by Student's *t* test.

RESULTS

Thirteen (25.5%) out of the 51 intact rats died during the 48-h period after the onset of ischemia and 17 (33%) rats died within 1 month. Nineteen (29%) out of the 65 SP(3-4)-treated animals died within 48 h and 25 (38%) within 1 month. During the acute (48 h) period of ischemia, mortality was minimal in intact rats with the active type of behavior, maximal among rats with the intermediate type, and medium among those with the passive type (Fig. 1, *a*), these differences being smoothed in SP(3-4)-treated rats (Fig. 1, *b*). After administration of SP(3-4), mortality during the acute period of CHB (48 h) tended to decrease among rats with intermediate behavior (21% *vs.* 54% in intact rats, $p<0.1$) and to increase among rats with active behavior (33% *vs.* 0%, $p=0.08$). One month after the onset of CHB, SP(3-4) insignificantly reduced mortality among rats with passive behavior (21% *vs.* 40% among intact rats), did not change mortality among rats with intermediate behavior (42% *vs.* 54%), and significantly increased it among rats with active behavior (58% *vs.* 11%, $p=0.04$). While most of the intact rats with severe neurological disorders (7-24 points) were from the groups with the passive and intermediate types of behavior (Fig. 2, *a*),

TABLE 1. Neurological Status of Rats (as Assessed in Scores) after Bilateral Ligation of the Common Carotid Arteries

Parameter	Score
Respiration:	
normal	0
increased/decreased rate	2
with participation of accessory muscles, irregular	4
Ptosis:	
none	0
unilateral	1
bilateral	2
Tone of limb muscles:	
normal	0
slightly increased	1
increased	2
absent	4
Convulsions:	
none	0
clonic	2
tonic	4
Response to external stimuli:	
normal	0
depressed	2
exaggerated and inappropriate	2
absent	4
Behavioral reactions:	
normal	0
hyperactivity, aggressiveness	2
hypoactivity	2
could not be determined due to gravity of condition	6
External appearance:	
groomed	0
ungroomed	1

Note. 25 points: death; 7-24 points: poor status; 3-6 points: intermediate status; 0-3 points: near-normal status.

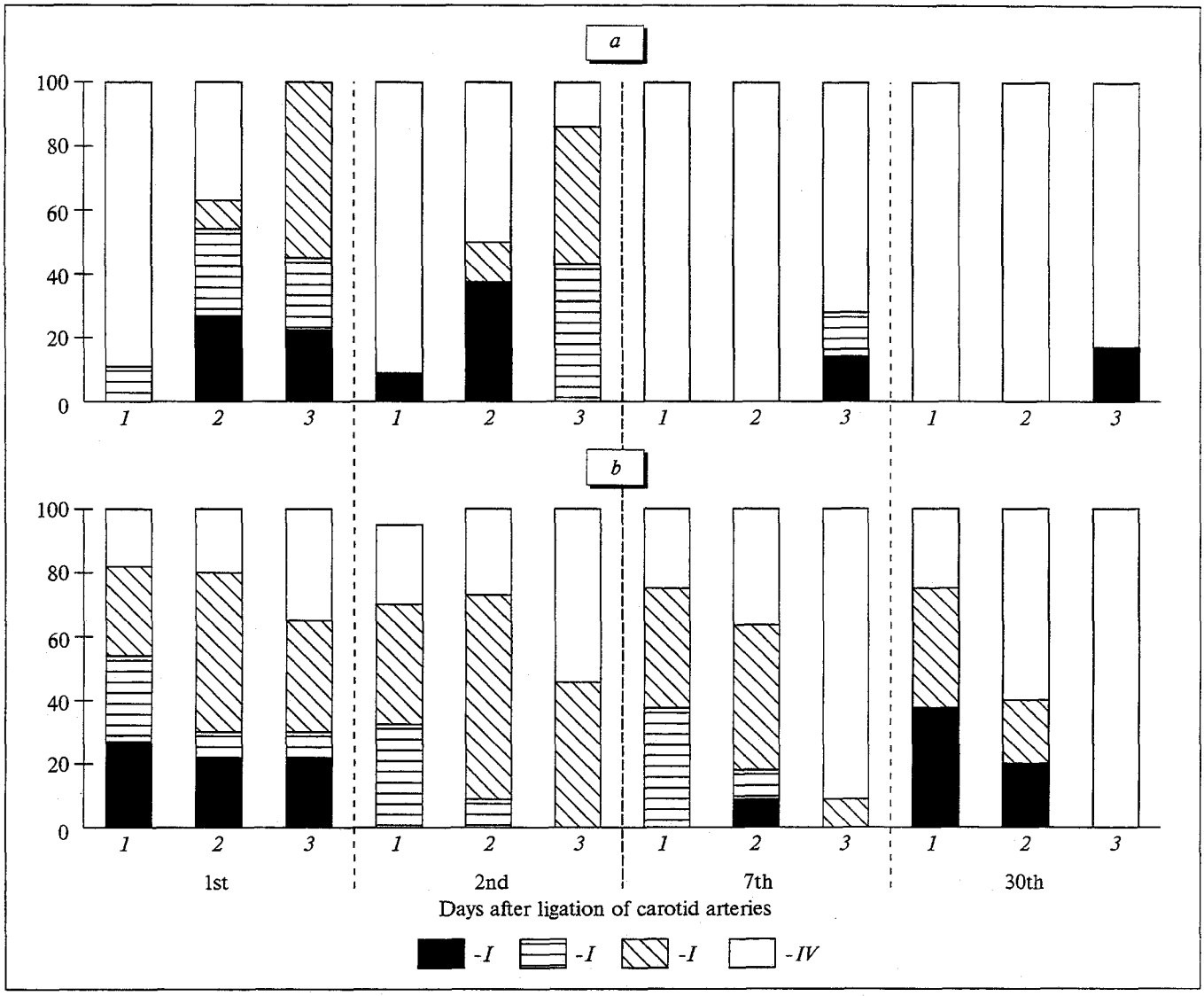

Fig. 2. Neurological status of rats with different types of behavior at different times after the onset of cerebral ischemia without (*a*) and with (*b*) administration of SP(3–4). *I*) death (25 points); *II*) poor status (7–24 points); *III*) intermediate status (3–6 points); *IV*) near-normal status (<3 points). Ordinate: number of animals with a particular neurological status, %.

most of SP(3-4)-treated rats with such disorders were from the groups with the active and intermediate types (Fig. 2, *b*). Among the rats with the passive type of behavior the number of animals with severe neurological symptoms decreased after administration of SP(3-4) (0% *vs.* 43%, $p<0.05$) and the number of symptom-negative animals increased (35% *vs.* 0%, $p< 0.05$, Fig. 2, *b*). By contrast, among the rats with the active type of behavior the number of symptom-positive animals increased (from 11% to 63%, $p< 0.05$) and the number of symptom-negative animals decreased (from 89% to 18%, $p<0.001$). Whereas among the intact rats only those with passive behavior died 7 and 30 days after the onset of ischemia (Fig. 2, *a*), animals with active and intermediate behavior died among the SP(3-4)-treated rats (Fig. 2, *b*). Previously, we demonstrated that SP significantly reduces mortality during the acute phase of CHB (48 h) in rats with a passive type of behavior, insignificantly reduces it in rats with an intermediate type, and insignificantly increases it in rats with active behavior [7]. Comparison of the effects of SP and SP(3-4) on the mortality of rats with different types of behavior showed that SP(3-4) has a similar but weaker positive effect on the resistance to CHB in rats with passive behavior and a similar but stronger negative effect in rats with active behavior. Since the similarity between the effects of SP and SP(3-4) manifested itself only when the effect of SP(3-4) on the "one-month mortality" was compared with the effect of SP on the "48-h mortality," it can be

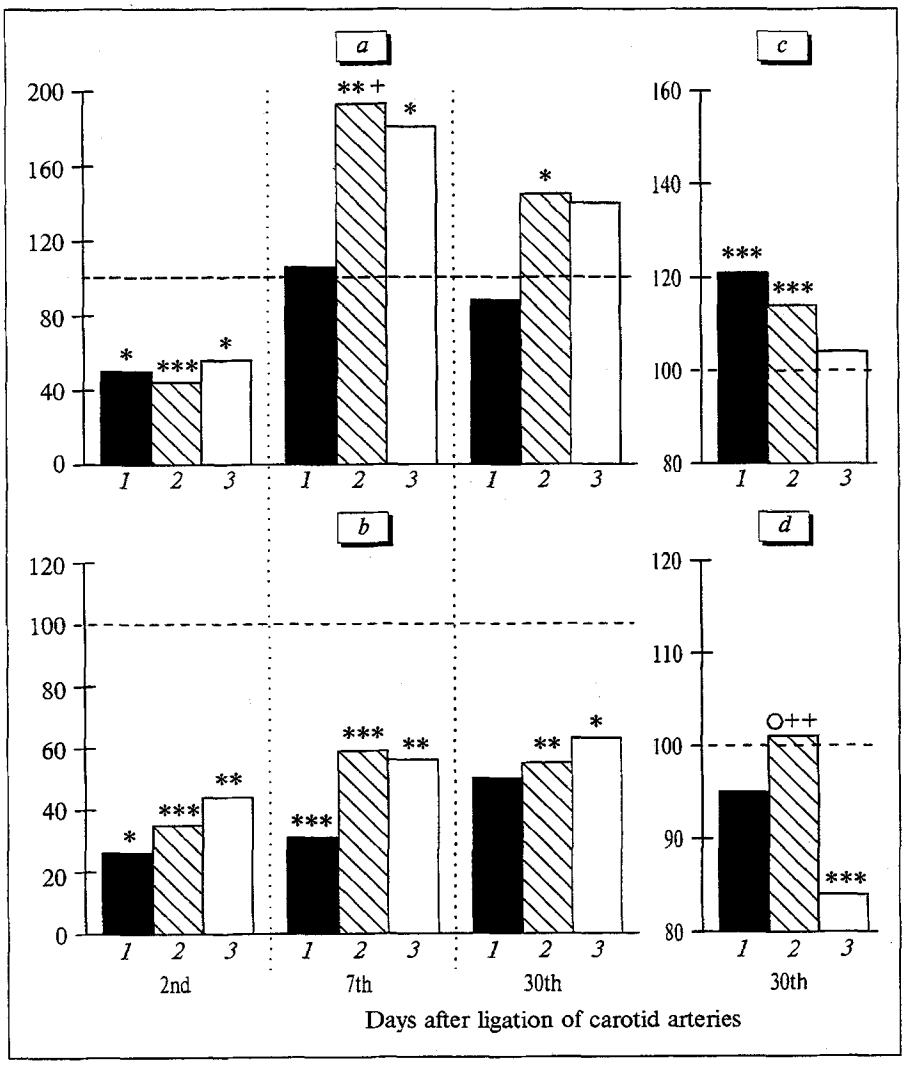

Fig. 3. Changes in the number of squares crossed in the open field test (*a, b*) and time of passive floating (*c, d*) in Porsolt's test among rats with different types of behavior at different times after the onset of brain ischemia without (*a, c*) and with (*b, d*) administration of SP(3–4). Data are expressed as a percentage of the baseline value which is taken as 100% (broken line). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared with the baseline, $^{+}p<0.05$, $^{++}p<0.01$ between groups *2* and *3*, and $^{\circ}p<0.01$ between groups *1* and *2*.

concluded that the effect of SP(3-4) on the CHB resistance of rats with active and passive behavior is similar to that of SP but slower and of an opposite direction. Although SP(3-4) did not significantly reduce mortality during the acute period of CHB among rats with the passive type of behavior, similarly to SP [5] it improved the neurological status of these rats and prevented their death at later times. An increase in motor activity on day 7 after ligation of the common carotid arteries was observed in rats with passive (from 45.1±8.5 to 81.5± 15.7, $p<0.05$) and intermediate (from 67.2±5.1 to 129.7±10.1, $p<0.01$) behavior. In rats with active behavior, brain ischemia in this period did not differ significantly from the baseline level (88.4±7.3 prior to ischemia and 93.9±21.4 after it, $p>0.1$) (Fig. 3, *a*, day 7). SP(3-4) not only prevented the development of posthypoxic hyperactivity, as did SP [5], but also significantly reduced motor activity in all rats compared with the baseline level (Fig. 3, *b*, day 7). One month after the onset of CHB, depression evaluated in Porsolt's test was increased to various degrees in all rats (Fig. 3, *c*). Similarly to SP [5], SP(3-4) abolished this change in behavior (Fig. 3, *d*).

Thus, not only the entire SP molecule consisting of 11 animo acids [5,7] but also its two-amino acid fragment has a positive therapeutic effect, albeit one that is less pronounced than that of SP. In other words, these results confirm the findings that the effects of SP and of its N-terminal fragments are similar [2,10,12]. Selectivity of the effects of SP and SP(3-4) is another similarity between these compounds. For example, like SP [5], SP(3-4) increases the resistance to CHB in rats with passive behavior, improving their neurological status and preventing the development of hyperactivity on day 7 after the onset of brain ischemia associated with postischemic damage to the brain [9]. SP(3-4) elicits a weak positive effect in rats with an intermediate type of behavior, in which it prevents posthypoxic hyperactivity, and, conversely, lowers the

CHB resistance of rats with active behavior, increasing their mortality and aggravating the neurological status. It can be speculated that investigation into the effects of various fragments of SP on the resistance to CHB will open up new prospects in the search for new antiischemic preparations with a selective therapeutic effect on individuals with a certain type of behavior, which may bring us closer to solving one of the most crucial problems in modern medicine: that of treating the individual rather than the disease.

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