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Effects of Angiotensin II on Behavioral Responses of Defensive Burying Paradigm in Rats

AKIRA TSUDA,[1] MASATOSHI TANAKA,[2] VASIL GEORGIEV[3] AND HIROYUKI EMOTO

Department of Pharmacology, Kurume University School of Medicine, Kurume 830, Japan

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TSUDA, A., M. TANAKA, V. GEORGIEV AND H. EMOTO. *Effects of angiotensin II on behavioral responses of defensive burying paradigm in rats.* PHARMACOL BIOCHEM BEHAV **43**(3) 729–732, 1992.—The effects of angiotensin II (ATII) administered intracerebroventricularly in male Wistar rats in doses of 0.1, 0.5, and 1.0 μg, as well as of ATII (1.0 μg) + saralasin (SAR, an analog ATII) (5.0 μg), on behavioral responses of the defensive burying paradigm were studied. ATII-treated animals displayed significantly less defensive burying behavior (less time spent in defensive burying and less frequent burying than in vehicle-treated rats) in a dose-dependent manner. SAR at a dose of 5 μg did not affect burying behavior significantly; it also did not modify the inhibition effects of ATII on behavioral responses of the defensive burying test. These results provide evidence that ATII can exert anxiolytic actions on central transmitter systems mediating conditioned fear-related behaviors (i.e., defensive burying). The present study suggests that the defensive burying animal model is a rather sensitive test fulfilling the pharmacological criteria of dose-dependent sensitivity for studying the central effects of neuropeptides (e.g., ATII).

Conditioned defensive burying Angiotensin II Saralasin Anxiety/fear Rat

OVER the past few years, it has been demonstrated that the octapeptide angiotensin II (ATII), administered ICV in mice, markedly alters seizure susceptibility and aversively motivated behavior. For example, ATII increases the threshold of seizures induced by chemical convulsants [pentylenetetrazol (PTZ), bicuculline, picrotoxin] (7) and by electroshock (6). Also, ATII facilitates memory of active avoidance conditioning/learning (12). ATII applied together with GABA, muscimol, and aminooxiacetic acid increases the PTZ seizure threshold-elevating effects of these GABAergic drugs; the effect is antagonized by bicuculline (11). Furthermore, ATII potentiates the anticonvulsant effects of GABA and diazepam on PTZ- and 3-mercaptopropionic acid-induced seizures when the drugs are applied at doses that do not significantly influence seizures (10). Moreover, ATII decreases the intensity of seizures in PTZ-kindled mice (9).

Because ICV ATII potentiates the anticonvulsant actions of diazepam and GABAergic agents and facilitates retention of active avoidance responses, it is possible that ATII may exert similar actions on central transmitter systems mediating fear-related behavior. It is worthwhile to ask, therefore, whether ATII may affect fear conditioning and/or exert anxiolytic effects. The following experiment was carried out to examine the effect of ATII on defensive burying in rats. Rodents use bedding material to bury noxious objects. In the shock-probe/defensive burying paradigm, rats are shocked through an electrified probe, whereafter they use an active strategy, namely, the pushing of bedding material toward or over the probe (defensive burying), to cope with the stress (14).

The defensive burying paradigm might represent an appropriate model for studying neuronal mechanisms of anxiety and its pharmacological analysis in animals (17–19). Anxiolytic drugs such as diazepam selectively suppress defensive burying behavior in a dose-dependent fashion, whereas the anxiogenic compound yohimbine (an α_2-adrenoceptor antagonist) potentiates this behavior (20) and causes anxiety and increased noradrenergic function in humans (1). We were therefore interested in determining whether ATII would influence defensive burying. It was expected that ATII would inhibit behavioral responses of the conditioned defensive burying paradigm if this compound has anxiety-modulated properties. Saralasin (SAR), an analog ATII, was also applied alone and in combination with ATII to examine if this drug

[1] Current address: Department of Human Sciences, Kurume University, Miimachi, Kurume 830, Japan.
[2] To whom requests for reprints should be addressed.
[3] Visiting Professor on leave from the Department of Experimental Pharmacology, Institute of Physiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria.

would produce partial agonistic actions or antagonistic actions against ATII at a dose previously shown to be efficacious in another behavioral paradigm (7).

METHOD

Subjects

Subjects were 48 naive male Wistar rats about 9 weeks of age (weighing 200–230 g) at the beginning of the experiment, obtained from Kyudo, K. K. (Kumamoto, Japan). Animals were housed in groups of four in standard propylene cage with food and water available on a free-feeding basis. Room temperature was controlled at 24 ± 1°C (relative humidity 50 ± 10%), and daylight conditions were artificially maintained between 0700 and 1900 h. All experimental procedures were carried out between 1300 and 1600 h.

Surgery

To administer the drugs ICV, the cannula implantation into the lateral cerebral ventricle was performed under pentobarbital (40 mg/kg, IP) anesthesia according to the method of de Wied (3). A middleline incision was made along the scalp, and the skin and underlying fascia were reflected bilaterally. A burr hole was drilled in the bone situated above the right lateral ventricle (0.5 mm posterior to bregma and 1.5 mm lateral to the midsagittal suture). Cannulae were made by poly(vinyl chloride) tubing. Stainless steel anchoring screws and dental cement served to secure the cannula in place. The top of the cannula terminated in the ventricle (−4.5 mm below the dura mater). Correct placement of the cannula was verified 24 h later by pushing a stillette into the tube and watching for cerebrospinal fluid. Rats with blocked cannulae were excluded from the study. Animals were allowed 5 days to recover.

Apparatus

A rectangular, transparent, acrylic plastic box (45 × 30.5 × 44 cm) was used as the testing chamber. The floor of the chamber consisted of 5 cm of white flake bedding material (Charles River Japan, Inc., Atsugi, Kanagawa). A white carbonate prod (6.5 × 1.2 cm) that was wrapped with two uninsulated wires was mounted in the center of an end wall of the testing chamber, 2 cm above the bedding material. The aversive stimulus generated by a shock generator-scrambler (SGS-001, Muromachi Kikai Co., LTD. Tokyo) was delivered to the wires of the shock prod.

Drugs

ATII (human form, synthetic; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.01 N acetic acid and kept in aliquots at −70°C until the day of the experiment. SAR ([Sar^{1}Ala^8]ATII) acetate (Sigma Chemical Co.) was also dissolved with 0.01 N acetic acid and kept under the same conditions as ATII.

Procedure

Habituation Session. Subjects were exposed to the test chamber without the shock prod for one 30-min habituation session on each of 3 consecutive days. On day 4, the shock prod was attached to the chamber and rats were placed individually in the center of the chamber for 10 min without giving shock when the rat touched the shock prod (burying baseline period). The bedding was cleaned of feces and smoothed to a uniform depth of 5 cm after each habituation and test trial.

Test Session. On day 5, rats were assigned to the following treatment groups (n = 8 in each group) on the basis of the duration of spontaneous burying displayed in the baseline period: vehicle; ATII 0.1, 0.5, 1.0 μg; SAR 5.0 μg; ATII 1.0 μg + SAR 5.0 μg and injected ICV by 10-μl Hamilton syringe. A 5-μl injection (1 μl isotonic saline, 1 μl drug solution, and 3 μl isotonic saline for flushing the injection volume) was delivered over 60 s; the syringe was kept with the needle remaining in place for an additional 30 s to allow for drug diffusion into the ventricle. Ten minutes after ATII or SAR (5 min SAR pretreatment then is followed by ATII for the combination condition), subjects were placed individually into the chamber with the shock prod and the 10-min test was performed. A 5-μl injection (1 μl isotonic saline, 1 μl 0.01 N acetic acid, and 3 μl isotonic saline for flushing) was delivered over the same time in the vehicle control group.

Behavioral Measurements. The behavioral observation and quantification used in the present experiment were similar to those used in previous investigations (20,21). The defensive burying-related responses were observed by two independent observers, using standard timers and event counters, in terms of the number of contacts with the shock prod, the latency to initiate the first defensive burying (latency of burying), frequency of burying episodes, and amount of time spent in burying (duration of burying). The defensive burying behavior was defined as a series of stereotyped sequences of the rat moving toward the shock prod, pushing and spraying a pile of bedding material at the prod with rapid movements of the snout and forepaws (14). The number of approach-avoidance responses, defined by a rat oriented toward the shock prod in an extended position and suddenly withdrawing from it, were also observed. At the end of each session, the height of piles of bedding within 10 cm of the prod was measured.

Data Analysis

Data were analyzed by a one-way analysis of variance (ANOVA) and subsequent Tukey's honestly significant difference (HSD) pairwise multiple comparisons.

RESULTS

Figure 1 depicts the duration and number of defensive burying responses during the test session. A one-way ANOVA (vehicle, ATII 0.1, 0.5, and 1.0 μg) of burying-duration data revealed a significant main dose effects of ATII, $F(3, 28) = 7.08$, $p < 0.01$. Also, the one-way ANOVA (vehicle, ATII 1 μg, SAR 5 μg, and ATII 1 μg + SAR 5 μg) of the burying-duration data revealed a significant main drug effect, $F(3, 28) = 4.13$, $p < 0.05$. Tukey's HSD posthoc comparisons ($\alpha < 0.05$) indicated that as compared with vehicle rats ATII- and ATII + SAR-treated rats were engaged in less defensive burying. The one-way ANOVAs of the number of defensive episodes also yield a significant main dose effect of ATII, $F(3, 28) = 16.41$, $p < 0.01$, and a significant main drug effect, $F(3, 28) = 8.15$, $p < 0.01$, respectively. Tukey's HSD posthoc comparisons ($\alpha < 0.05$) indicated that ATII- and ATII + SAR-treated rats showed less frequent defensive burying than did vehicle-treated rats. SAR alone, although not significant, also tended to decrease the duration and number of burying responses. SAR at the dose used, administered before ATII, was not able to significantly affect the effects of ATII.

Table 1 summarizes the various measures of defensive burying-related responses as a function of drug treatments. As for the number and latency of touches with the shock prod, neither dose effect of ATII nor drug effect were significant. With respect to latency of burying, both dose effect of ATII

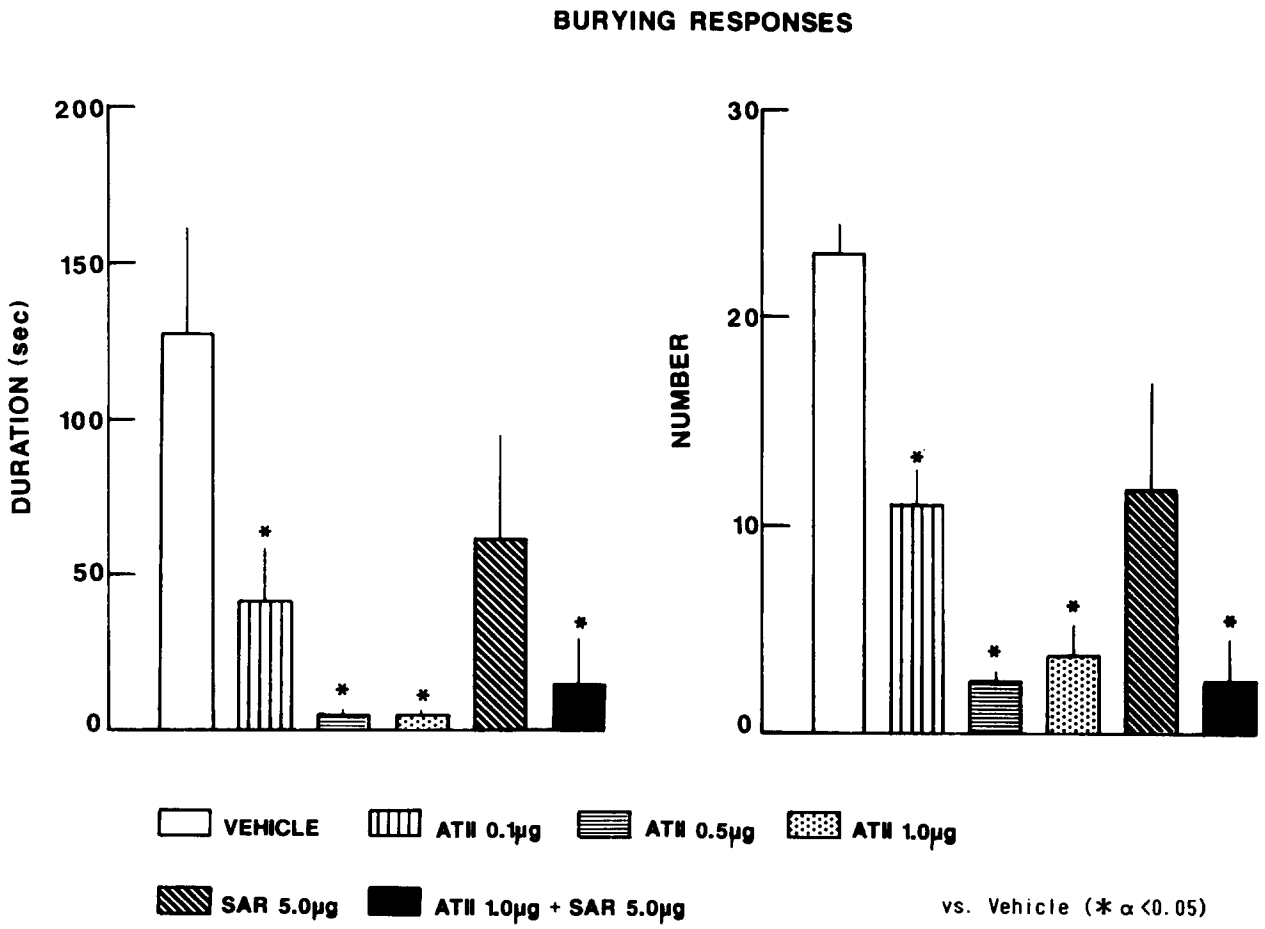

FIG. 1. Mean (±SEM) duration (left) and number (right) of defensive burying responses in test session for rats given an ICV injection of vehicle, ATII 0.1, 0.5, and 1.0 μg, SAR 5.0 μg, or ATII 1.0 μg + SAR 5.0 μg. Significantly different from vehicle ($\alpha < 0.05$; Tukey's HSD test).

and drug effect were significant, $F(3, 28) = 3.09$, and 6.49, $p < 0.05$, respectively. With respect to height of pile and number of approach-avoidance responses, ATII significantly decreased them, $F(3, 28) = 5.00$, and 3.63, $p < 0.05$, respectively. Combination with SAR significantly or almost significantly decreased the height of pile, $F(3, 28) = 3.07$, $p < 0.05$, as well as the number of approach-avoidance responses, $F(3, 28) = 2.68$, $p < 0.1$. There were no significant differences among the ATII 1 μg, SAR 5 μg, and ATII 1 μg + SAR 5 μg in terms of any burying-related responses.

DISCUSSION

The present study shows that the octapeptide ATII administered ICV in rats suppresses defensive burying-related behav-

TABLE 1

SUMMARY (±SEM) OF CONDITIONED DEFENSIVE BURYING-RELATED RESPONSES

Treatments	Latency of Burying (seconds)	Height of Pile (cm)	Number of Approach-Avoidance Responses	Number of Touches	Latency of Touches (seconds)
Vehicle	101.5 ± 28.3	2.5 ± 0.3	5.1 ± 0.7	2.4 ± 0.6	12.0 ± 3.1
Angiotensin II					
0.1μg	148.6 ± 48.6	1.6 ± 0.7	4.5 ± 0.7	2.9 ± 0.3	13.2 ± 1.7
0.5μ	267.4 ± 50.6	0.3 ± 0.1*	2.2 ± 0.5*	2.9 ± 0.3	22.7 ± 9.0
1.0μg	314.2 ± 74.0*	0.5 ± 0.3*	3.0 ± 0.7	2.4 ± 0.4	14.7 ± 4.2
Saralasin 5.0μg	275.4 ± 90.2	1.7 ± 0.8	4.2 ± 1.4	2.4 ± 0.5	13.7 ± 5.9
Angiotensin II 1.0μg + saralasin 5.0μg	540.2 ± 55.8*	0.6 ± 0.5*	1.6 ± 0.5*	2.9 ± 0.4	25.4 ± 7.7

*Significantly different from vehicle ($\alpha < 0.05$; Tukey's HSD test).
Each value indicates the mean ± SEM of eight rats.

iors. The results indicate that pretreatment with ATII reduces the duration and frequency of defensive burying responses in a dose-dependent manner. Although not so evident, ATII significantly reduces the height of pile and number of approach-avoidance responses.

The study also shows that SAR at a dose of 5 μg produces effects on defensive burying behavior similar in magnitude to the 0.1-μg dosage of ATII. As a peptide analog of ATII, SAR retains substantial partial agonistic properties on AT receptors (16), and at the dose applied it produces agonistic actions in the defensive burying paradigm. Obviously, this dosage of SAR was not sufficient to block the effects of ATII on defensive burying behavior. In another study of ours (12), SAR also behaved as an ATII agonist, thus inducing effects similar to facilitation of retention of active avoidance by ATII. Yet, at a larger dose SAR decreases the retention-improving effect of ATII and should be envisaged as an ATII antagonist.

ATII apparently produces a complex behavior; it plays a major role in the regulation of drinking behavior, vasopressin secretion, etc. (4,13). Thus, when administered centrally into sexually experienced male rats ATII suppresses sexual motivation (increased latency to the initiation of copulatory behavior) and increases the number of intromissions preceding ejaculation. These effects appear at a lower dose than that required to stimulate drinking in this specific situation (2). It might be assumed that this is also the case in the present experiment. To produce dipsogenic responses and the present effects on defensive burying, the primary event might be the binding to ATII receptors (5) or to its type 1 or type 2 receptors in specialized parts of the brain (15). All these brain sites, accessed via the ICV route, might take part in the effects of ATII on defensive burying behavior. Especially, it was reported that the type 1 ATII receptors play a role in cardiovascular control and stress (22,23). It might be also assumed that ATII realizes its effects on defensive burying behavior by interaction with neurotransmission in the responsible areas for this type of behavior. We have data to show that GABAergic and dopaminergic transmissions participate in the mechanism of the coping-facilitating effects and seizure threshold-increasing effects of ATII (6,8,10,11).

In conclusion, the present study has provided evidence that ATII influences the defensive burying-related behavior by suppressing mainly the number and duration of burying responses, although these effects of ATII were not significantly affected by a moderate dose of SAR. The results from this study suggest that ATII may suppress defensive burying by reducing the animals' fear motivation. Further studies of interactions with diazepam and GABAergic drugs will be necessary to establish a behavioral specificity for the anxiolytic effects of ATII in the defensive burying paradigm.

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