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The contribution of AT_1 and AT_2 angiotensin receptors to its cognitive effects

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Abstract. In this study I attempted to assess, in rats, the role of AT_1 and AT_2 angiotensin receptor subtypes in the phenomenon of improved learning and memory after an intracerebroventricular (icv) injection of angiotensin II (Ang II) and Ang II (3-7). Selective AT_1 (losartan, 1 mg) or AT_2 (CGP 42112 A, 2 μg) receptor antagonist was dissolved in 2 μl of saline and given to the left cerebral ventricle 5 min before 1 nmol Ang II or Ang II (3-7) injected in the same volume of saline to the right ventricle. Consequently, there were 9 experimental groups which underwent 3 memory oriented and 3 auxiliary tests. Ang II and Ang II (3-7) significantly improved retention of the passive avoidance and recognition memory. These effects were abolished by losartan or CGP 42112 A . Better, after Ang II and Ang II (3-7), acquisition of conditioned avoidance responses was unchanged by losartan and abolished by CGP 42112 A. None of the treatments significantly changed rats motor behaviour in open field. Losartan as well as CGP 42112 A abolished significant enhancement of apomorphine (1 mg/kg, ip) stereotypy caused by Ang II and Ang II (3-7). The results suggest considerable involvement of AT_1 and AT_2 angiotensin receptors in the cognitive enhancement produced by angiotensins.

Key words: angiotensins, angiotensin receptor subtypes, memory, motor behaviour, rat

INTRODUCTION

Facilitating learning and enhancing memory effects of the hypertensive octapeptide angiotensin II (Ang II) were first described by Baranowska et al. in 1983. This early study showed that the peptide given intercerebroventricularly (icv) at the dose of 1 μg (about 1 nmol) markedly and significantly increased the rate of acquisition of conditioned avoidance responses (CARs) by rats. The effect persisted for several days and was dose-dependent as 2 μg of Ang II caused higher increase of the rate of CARs acquisition than 1 μg. However, 5 μg of Ang II given icv did not stimulate learning giving overall picture of the inverted U-shaped curve of the dose-effect relationship, typical for many peptides (Kovacs and De Wied 1994). Interestingly, Sar-1, Ile-8 angiotensin II (sarile), the potent Ang II receptor antagonist also, similarly to Ang II itself, accelerated CARs acquisition by rats.

Basing on these studies we concluded that the cognition enhancing activity of Ang II and its congeners must depend on the 2-7 amino acid sequence in its molecule because substitution of the first and eighth position, as it is in sarile, caused practically no change in the psychoactivity of the peptide (Wiśniewski and Braszko 1985). In the further studies we examined Ang II fragments to support this notion and found that 2-8 and 3-8 fragments of Ang II named Ang III and Ang IV, respectively, were fully active at enhancing memory in rats, thus confirming the above hypothesis and expanding it in that Arg-2 is also negligible for the psychoactivity of angiotensins (Braszko et al. 1987). Soon it became clear that even shorter Ang II fragment, the pentapeptide Ang II (3-7), was also equipotent with the parent hormone at facilitating learning and recall in rats (Braszko et al. 1991). The equal psychotropic activity of Ang II and Ang II (3-7), despite the 10^4 times lower affinity of the fragment than the Ang II itself (Mann et al. 1981) to the AT_1 and AT_2 angiotensin receptors, pointed to the nonreceptor mediated mechanism of the cognitive enhancement caused by angiotensins (Braszko et al. 1988).

At the end of eighties AT_1 and AT_2 angiotensin receptors were identified and their specific antagonists were developed (Chiu et al. 1989, Whitebread et al. 1989, Stephenson et al. 1992, Stroemberg et al. 1993). Therefore it became possible to verify more directly our hypothesis that the cognition enhancing effects of angiotensin peptides are relatively independent of their interaction with AT_1 and AT_2 angiotensin receptors.

METHODS

We pretreated rats with the specific AT_1 (losartan, 1 μg ~2 nmol) or AT_2 (CGP 42112 A, 2 μg ~1,5 nmol) angiotensin receptor inhibitors and then tested them for their ability to express the enhancement of learning and memory after an icv administration of Ang II and Ang (3-7). We also checked for another, typical for these peptides, behavioural alterations. The dosage of the antagonist was adjusted to ensure, on the molecular basis, the complete antagonism of our doses of Ang II and Ang II (3-7), which in both cases was 1 nmole. There were two sets of behavioural procedures used, one cognition-oriented, and the other designed to control for the aspects of behaviour which may unspecifically change the expression of cognitive alterations. The cognition-oriented procedures included: (1) retrieval of passive avoidance (Ader et al. 1972), (2) learning of active avoidance (Baranowska et al. 1983), and (3) delayed object recogniton (Cavoy and Delacour 1993, Braszko et al. 1995).

The auxiliary control tests were: (4) motor activity of animals in open field, to control for the influence of rats motor performance by our treatments; (5) the behaviour of rats in an elevated "plus" maze (Pellow and File 1986), to control for the anxiogenic / anxiolytic properties of our drugs, and (6) modification of the stereotypic behaviour produced by apomorphine, a nonselective D_1 / D_2 dopamine receptors agonist (Ernst and Smelik 1966), to check for the influence of angiotensins and its AT1 and AT2 receptor antagonists on the dopaminergic reactivity. It is widely accepted that the dopaminergic system is critically involved in the cognitive processes (Simon et al. 1980, Wiśniewski and Braszko 1984, Decker and McGaugh 1991).

RESULTS AND DISCUSSION

The results of these experiments will be published in detail soon and I will hereby give only a preliminary account of the data which, however, will allow the reader to make a reasonably precise view on the importance of AT_1 and AT_2 angiotensin receptors for the cognition enhancing activity of angiotensins.

The inhibition of AT_1 receptors by losartan virtually abolished Ang II- and Ang II (3-7)- induced about sevenfold increase of the effectiveness of recall of the passive behaviour allowing the animal to avoid adverse electric stimulation (Karwowska 1995, Kułakowska 1995). CGP 42112 A significantly, but much less than losartan, diminished facilitation of retrieval of memory produced by Ang II and Ang II (3-7) (unpublished results). Losartan, as well as CGP 42112 A, did not, by itself, influence the passive avoidance. Conditioned active responding is different from the passive avoidance procedure and it is testing learning capability. In order to avoid painful electric stimulation the animal has to move in a shuttle - like manner between the two compartments in response to an unconditioned stimulus (buzzer). Losartan did not change the rate of acquisition of CARs in control as well as in Ang II- or Ang II-(3-7)-pretreated rats (Karwowska 1995, Kułakowska 1995). CGP 42112 A however, markedly diminished pronounced stimulatory effects of both peptides on learning (unpublished results). In the third cognition-oriented experimental paradigm, the object recognition, no aversive stimulation was used. The animal was aquainted with the environment and then, after a delay, it was tested for the recognition of novel, previously absent from this environment object. The recognition was expressed in longer exploration of the new object by an animal driven by the natural trait of novelty-seeking . Losartan totally abolished, about twice prolonged by Ang II and Ang II (3-7), time of exploration of the new objects by rats (Braszko et al. 1994). The effect of the AT_2 receptor blockade by CGP 42112 A was almost identical (unpublished results).

Summarising the results obtained in the cognition-oriented experiments we found diminution of clear, learning and memory enhancing, effects of Ang II and Ang II (3-7) by blocking both AT_1 or AT_2 angiotensin receptors. The only exception was the lack of influence of AT_1 receptor inhibition on CARs acquisition. It therefore appears that indeed, AT_1 and AT_2 angiotensin receptors are important in mediating stimulatory effects of angiotensins on learning and memory.

There are, however, few issues which have to be solved before such a conclusion is accepted. Firstly, how losartan and CGP 42112 A change motor performance of animals. The importance of that issue is difficult to overrate when one remembers that the whole reasoning concerning memory is based on the observation of motor behaviour. The careful quantification of the animals locomotor exploratory activity in open field did not, however, reveal any marked influence of neither the peptides nor blockers (Karwowska 1995, Kułakowska 1995). Square crossing was unchanged at all while rearing and bar approaches only tended to be increased and decreased, respectively, by the peptides and CGP 42112 A. These results point to the lack of motor influences of losartan and CGP 42112 A and to some decrease of exploration caused by the latter compound and therefore support our conclusion.

Secondly, do losartan and CGP 42112 A influence anxiety evoked by the behavioural avoidance procedures using electric foot shock. Such an influence could significantly bias the reasoning concerning memory. On the elevated "plus" maze losartan doubled the time spent by animals in open arms and almost doubled the number of open arms entries regardless of whether they were pretreated with the peptides or not (Karwowska 1995, Kułakowska 1995). These results only confirm anxiolytic properties of losartan described by Kaiser et al. (1992). The antianxiety properties of losartan can not, however, explain abolishment of the better retrieval of memory caused by Ang II and Ang II (3-7) because CGP 42112 A having identical effect on passive avoidance was completely inactive in changing the "plus" maze behaviour no matter

whether the rats were or were not subsequently treated with Ang II and Ang II (3-7) (unpublished results). It, therefore, appears that the modification of anxiety by the AT_1 and AT_2 angiotensin receptor blockers was not a major factor causing dissipation of cognition enhancing properties of Ang II and Ang II (3-7).

Thirdly, for several years we have been searching for changes in the central dopamine system being under the influence of angiotensins. Enhancement of the stereotype behaviour has consistently been shown after an icv administration of Ang II, its fragments, and analogues having at least Ang II (3-7) sequence within their molecule (Braszko and Wiśniewski 1976, 1988, 1990, Braszko et al. 1986, 1987, 1988, 1991, Hoły et al. 1992). All these peptides showed significant cognition enhancing properties that could imply a causal relationship between the stimulation of central dopamine neurotransmission and learning. The more so that the most at these peptides affected also the metabolism of dopamine in brain (Simonnet et al. 1981, Sumners and Philips 1983, Braszko et al. 1991) which, as already mentioned, is basic for the cognitive processes (Packard and White 1989, 1991, Sawaguchi and Goldman-Rakic 1991, McGurk et al. 1992, Bushnell and Levin 1993). The angiotensin fragments which did not contain complete Ang II (3-7) amino acid sequence like Ang II (3-5) and Ang II (3-6) (Własienko et al. 1989) or Ang II (1-6) and Ang II (2-6) (Holy et al. 1994) were inactive at both, intensifying stereotype behaviour and enhancing memory. Therefore, it was indispensable to check both our antagonists for their potential of influencing stereotype behaviour which results from the central dopaminergic stimulation. Losartan, the AT_1 antagonist, significantly reduced, and CGP 42112 A, the AT_2 antagonist, totally abolished significant increase of the stereotyped behaviour produced by Ang II and Ang II (3-7) (unpublished results). Dwoskin et al. (1992) found inhibition of the striatal dopamine neurotransmission by losartan and the present results are in line with this finding. WL-19, another potent AT_2 receptor antagonist, was also found to have an antistereotypic activity (Banks and Dourish 1991). On the contrary, p-NH_2Phe-6 Ang II, an agonist of the AT_2 receptors intensified stereotypy as well as facilitated recognition and retrieval of memory (unpublished results).

Thus, it appears that the antidopaminergic effects of both AT_1 and AT_2 angiotensin receptors blockers may be causally linked with their potential of preventing cognition enhancing activity of angiotensins. It is worth mentioning here that losartan also inhibits brain NK_3 tachykinin receptors (Chretien et al. 1994) which, on the other hand, may be involved in the cognitive processing through their ability to stimulate acetylcholine release (Laufer et al. 1985). Also, AT_4 angiotensin receptors, recently discovered by the Wright's group, and abundant in the brain structures related to memory (cortex and hippocampus), are thought to be responsible for the most cognition enhancing effects of angiotensin peptides (Wright et al. 1995).

In summary, it appears that, contrary to our expectations, both AT_1 and AT_2 angiotensin receptors are engaged in the cognitive enhancement produced by angiotensins, particularly in facilitation of learning and improvement of memory in rats. Some unresolved issues like what is the degree of this engagement in relation to the involvement of the AT_4 receptors or, even more interestingly, why effects of Ang II are diminished to the extent comparable to that of Ang II (3-7) in spite of 10000 times lower affinity of Ang II (3-7) than Ang II to the AT_1 and AT_2 receptors will undoubtedly be addressed in the subsequent studies.

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