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Involvement of Nitric Oxide Formation in the Action of Ramipril and Ramipril-Octil in an Inhibitory Avoidance Task in Mice

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ABSTRACT

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We tested (in an inhibitory avoidance test) ramipril and the n-octyl-ester of ramipril (RA-octil; Hoe 065), which was shown to have no significant influence on plasma-converting enzyme (CE) activity or blood pressure in spontaneously hypertensive rats. In addition, the influence of the specific NO-synthase inhibitor N^G-nitro-L-arginine (L-NNA) and the specific B2 antagonist Hoe 140 in combination with ramipril and RA-octil were tested. Ramipril and RA-octil showed a significant prolongation of step-through latencies. L-NNA and Hoe 140 by themselves showed no effects. The combination of ramipril and of RA-octil, respectively, with L-NNA or Hoe 140 did not produce any prolongation of step-through latencies.

Key words: Hoe 140, nitric oxide, N^G-nitro-L-arginine, memory

INTRODUCTION

Converting enzyme (CE, EC 3.4.15.1) is a dipeptidyl-carboxy peptidase that cleaves histidyl-leucine from the carboxyl terminus of angiotensin I (ANG I) to yield the octapeptide angiotensin II (ANG II) [Cushman and Cheung, 1971; Geiger, 1984; Severs and Daniels-Severs, 1973]. CE also cleaves C-terminal residues from a variety of other peptides including bradykinin, substance P, enkephalins, and neurotensin [Cascieri et al., 1984; Dorer et al., 1974; Erdös et al., 1978; Skidgel et al., 1984].

The octapeptide ANG II is known as the circulating effector peptide of the renin-

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angiotensin system. It is classically linked to the systemic control of blood pressure [Page and Bumpus, 1961; Peart, 1965]. On the other hand, ANG II was shown to have central effects [Felix and Schlegel, 1978; Severs and Daniels-Severs, 1973] and to be present in brain tissue [Fischer-Ferraro et al., 1971; Phillips et al., 1979]. Centrally administered ANG II was reported to disrupt learning and retention performance in rats [Morgan and Routtenberg, 1977].

In a histochemical study Haas et al. [1980] found an angiotensin-like (ANG II) immunoreactivity in the pyramidal cell layer of the hippocampal area CA1 and CA3 and in the stratum granulosum of the area dentata. Electrophysiological recordings revealed a rapid depolarizing action and an increase in firing rate, but no measurable change in membrane input resistance. Bath-applied ANG II increased the amplitude of population spikes evoked by stimulation of the Schaffer-collaterals or commissural fibers and induced multiple spikes. Haas et al. [1982] explained their results as an epileptic sign. As mentioned earlier, ANG II has been shown to disrupt learning, and it is well known that convulsions cause amnesia and that long-term potentiation (LTP), which is related to memory processes in the hippocampus, is lost following seizures [Hesse and Teyler, 1976].

Recent experiments indicate that nitric oxide (NO) is produced in the granule cells of the cerebellum in response to glutamate application [Garthwaite et al., 1988]. There are reasons to suggest an even more fundamental role for NO in altering synaptic efficacy within various brain regions in the adult as well as in synaptogenesis during development. Furthermore, a body of data has accumulated demonstrating that the synaptic changes of LTP result from the temporal correlation of presynaptic activity and postsynaptic depolarization [Bliss and Lynch, 1988]. Recently, Williams et al. [1989] have suggested that the signal from the postsynaptic site to the presynaptic site in LTP may be NO.

In this context it is interesting that CE inhibition leads to the accumulation and release of bradykinin in the vascular wall with subsequent formation of NO. In earlier experiments we tested [in an inhibitory (passive) avoidance test] ramipril [unpublished data] and the n-octyl-ester of ramipril (RA-octil) [Hock et al., 1989; Wiemer et al., 1989], which was shown to have no significant influence on plasma CE activity or blood pressure in spontaneously hypertensive rats. To understand the possible mechanisms of action, we therefore tested the influence of the specific NO-synthase inhibitor N^G-nitro-L-arginine (L-NNA) as well as the specific B2 antagonist Hoe 140 [Hock et al., 1991; Wirth et al., 1991] in combination with ramipril and RA-octil in this behavioral experiment.

MATERIALS AND METHODS

Test Procedure

Male mice of the NMRI strain (Hoechst Breeding Farm) weighing 20–25 g were used in the experiments. All animals were maintained on a 12 hr dark/light cycle (lights on 6 a.m.).

The test apparatus was a modified Jarvik step-through inhibitory avoidance box [Hock and McGaugh, 1985; Kopp et al., 1967] consisting of a small chamber connected to a larger dark chamber via a guillotine door. The small chamber was illuminated with a 7 W/12 V bulb. The mice were given an acquisition trial followed by a retention trial 24 hr later. In the acquisition trial a mouse was placed in the illuminated compartment at a maximal distance from the guillotine door, and the latency to enter the dark compartment was measured. Animals that did not step through the door within 90 sec (cut-off time) were not used. Immediately after the mouse had entered the dark compartment the door was shut automatically and an unavoidable footshock (FS: 1 mA; 1 sec) was delivered. The mouse was then quickly removed (within 10 sec) from the apparatus and put back into its home cage. The cut-off time on day 2 was 300 sec.

Ramipril was tested in a dose range of 0.03–10 mg/kg i.p. and RA-octil was tested in a dose range of 0.03–30 mg/kg i.p. The ramipril- and the RA-octil groups were treated 60 min

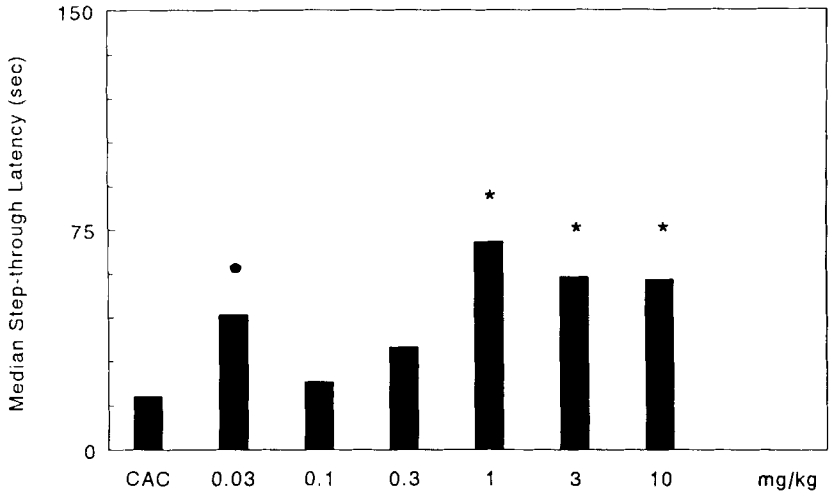

Fig. 1. Effects of ramipril (i.p.) on inhibitory avoidance in mice. CAC, control group (received vehicle only). *, $P < 0.05$, Kruskal-Wallis ANOVA.

prior to training and testing. In a second set of experiments L-NNA (tested doses: 0.03, 0.3, 3, and 30 mg/kg i.p.) and Hoe 140 (tested doses: 0.1, 0.3, and 1 mg/kg s.c.) were tested in this model. The L-NNA and the Hoe 140 groups were treated 30 min prior to training and testing. Scopolamine was given in an amnesic dose of 2 mg/kg s.c. 5 min prior to the acquisition test.

The behavioral experiments described here were carried out in accordance with the Tierschutzgesetz (Animal Protection Law) of the Federal Republic of Germany, i.e., they were approved in writing and supervised by state and town authorities enforcing the Tierschutzgesetz locally on behalf of the Federal Government.

Drugs

Ramipril, RA-octil, and Hoe 140 were synthetized at Hoechst AG and were dissolved in 0.9% saline containing 2% ethanol. (−)-Scopolamine hydrobromide and N^G-nitro-L-arginine (L-NNA) were purchased from commercial sources.

Statistics

The results are expressed as medians. The significance of differences was analyzed with the Mann-Whitney U-test [Sachs, 1984] and Kruskal-Wallis ANOVA [Sachs, 1984]. A probability of 0.05 was accepted as significant.

RESULTS

In the training trial the mice treated with scopolamine (CAC) showed (besides the amnesia) a slightly longer latency to enter the dark compartment in comparison to animals not treated with scopolamine. The median response latency on day 1 was 19 sec (average of all groups) and 26 sec for the CAC group (data not shown). The difference was not significant.

In all experiments the median retention latencies of the control groups (C-C) in the 24 hr retention test was 300 sec (cut-off time). The latencies of the drug groups (D-D) not given scopolamine were not effected by the drug administration, i.e., the median retention latency in these groups was 300 sec.

As shown in Figure 1, the CAC group showed a marked reduction of the median

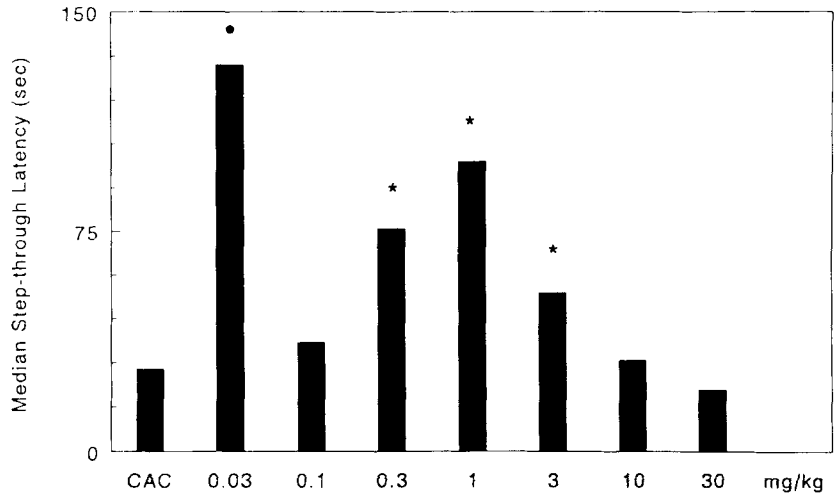

Fig. 2. Effects of RA-octil (i.p.) on inhibitory avoidance in mice. CAC, control group (received vehicle only). *, $P < 0.05$, Kruskal-Wallis ANOVA.

retention latencies (median latency: 18 sec) in comparison to the latencies of the untreated groups (C-C). The impairing effect of scopolamine on the retention latencies was significantly attenuated by ramipril given prior to the acquisition and retention tests (DAD) in doses of 0.03 mg/kg and 1–10 mg/kg i.p. ($P < 0.05$, Kruskal-Wallis ANOVA).

The RA-octil experiments showed that the impairing effects of scopolamine were significantly attenuated by this compound in doses of 0.03 mg/kg and 0.3–3 mg/kg i.p. ($P < 0.05$, Kruskal-Wallis ANOVA; Fig. 2).

As shown in Figures 3 and 4, L-NNA (0.03–30 mg/kg i.p.) and Hoe 140 (0.1–1 mg/kg s.c.) did not influence the effects of scopolamine. There was no attenuation or potentiation of the scopolamine effects.

In the combination studies L-NNA was tested in a dose of 3 mg/kg i.p. and Hoe 140 was tested in a dose of 1 mg/kg s.c. The retention-enhancing effects of ramipril and RA-octil were abolished in both combinations (Figs. 5–8). The slight increase in two groups given RA-octil/L-NNA (0.3/3 mg/kg and 10/3 mg/kg) was not significant.

DISCUSSION

Our findings indicated that ramipril and RA-octil [for details see Hock et al., 1989] enhanced retention in an inhibitory (passive) avoidance test after scopolamine amnesia. The active dose range of both compounds was comparable. The inverted U-shaped or the double bell-shaped dose-response curves seen in these experiments were comparable to those obtained with other memory-enhancing drugs and hormones [Bartus et al., 1980; Cumin et al., 1982; Fekete and deWied, 1982; Hock and McGaugh, 1985; Hock et al., 1988, 1989; McGaugh, 1985]. The reason for the lack of effectiveness of the higher doses was not clear. A possible explanation of the dose-dependent facilitation by ramipril and RA-octil was that these molecules have two different intrinsic activities. Similar results were described with ORG 2766, and the possibility that a molecule has two different intrinsic activities has been discussed [Fekete and deWied, 1982]. The type of dose-response curve also suggested that ramipril and RA-octil may have more than one type of mechanism, one seen at low doses and the other at

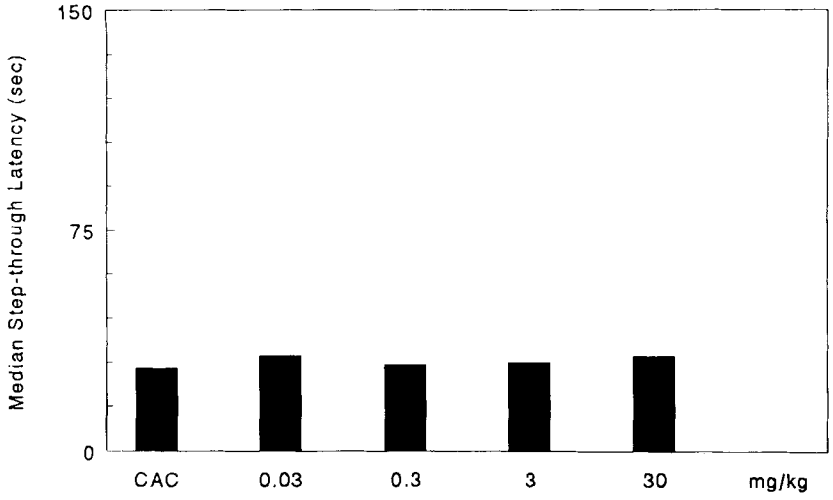

Fig. 3. Effects of L-NNA (i.p.) on inhibitory avoidance in mice. CAC, control group (received vehicle only).

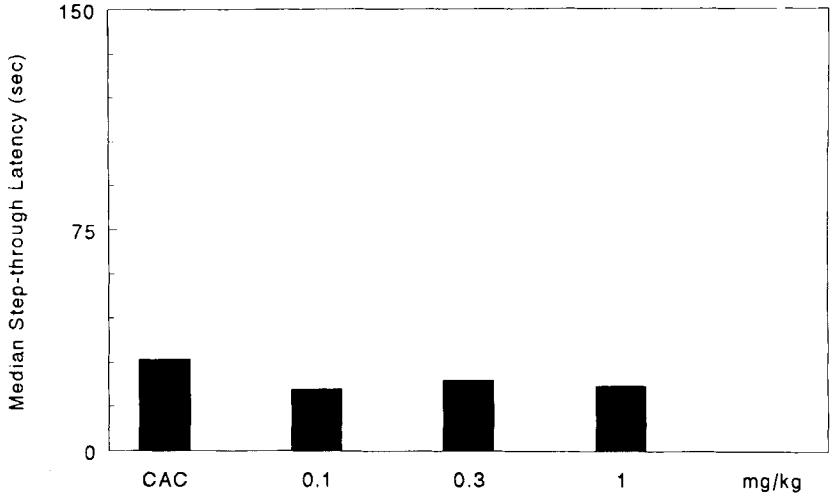

Fig. 4. Effects of Hoe 140 (s.c.) on inhibitory avoidance in mice. CAC, control group (received vehicle only).

high doses. Similar behavioral results have been described by Nabeshima et al. [1988] for the compound NIK-247, a 4-aminopyridine derivative.

The potent and selective NO-synthase inhibitor L-NNA [Dwyer et al., 1991; Lambert et al., 1991] had no influence on scopolamine-induced amnesia in this model. The same results were obtained using Hoe 140, a potent and long-acting bradykinin antagonist [Hock et al., 1991; Wirth et al., 1991]. However, the retention-enhancing effects of ramipril and RA-octil could be blocked by L-NNA as well as by Hoe 140.

This was in accordance with results of a recent study by Wiemer et al. [1991b], who demonstrated evidence of the formation of bradykinin by cultured endothelial cells from

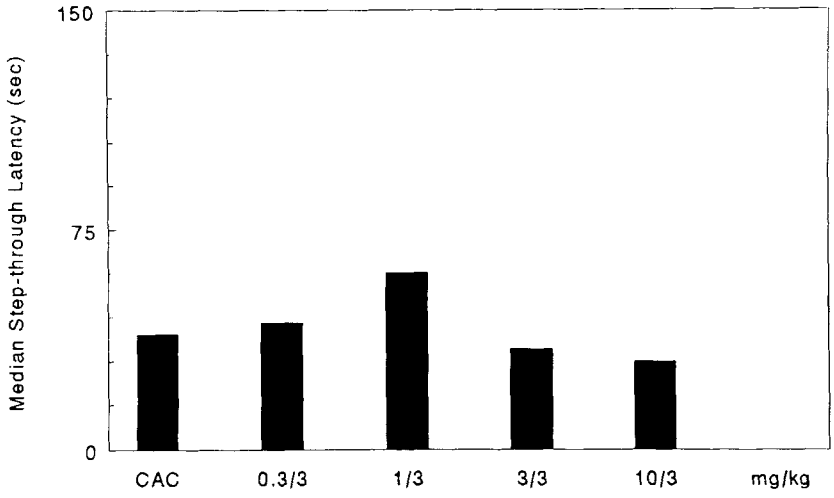

Fig. 5. Effects of the combination of ramipril (i.p.) and L-NNA (i.p.) on inhibitory avoidance in mice. CAC, control group (received vehicle only). Under each bar, the first number represents the dose of ramipril; the second represents the dose of L-NNA.

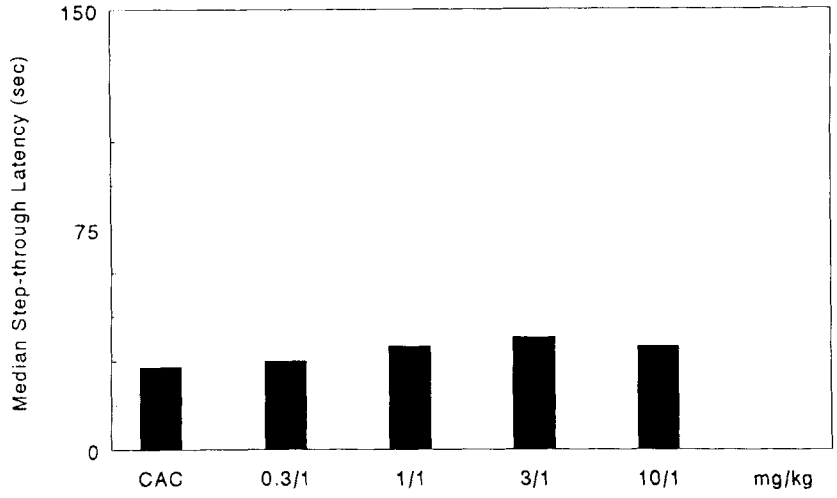

Fig. 6. Effects of the combination of ramipril (i.p.) and Hoe 140 (s.c.) on inhibitory avoidance in mice. CAC, control group (received vehicle only). Under each bar, the first number represents the dose of ramipril; the second represents the dose of Hoe 140.

bovine aorta. Martin et al. [1988] and Schini et al. [1990] showed an enhanced production of cyclic guanine monophosphate (cGMP) in endothelial cells in response to NO-releasing agonists such as bradykinin, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and calcium ionophore. It was shown that CE inhibitors stimulate the formation of NO and prostaglandin (PG)I_2 in endothelial cells, most likely by inhibiting the breakdown of endothelial-derived kinins. This was proved by using the selective B2-receptor antagonist Hoe 140, which abolished the enhanced NO and PGI_2 formation observed after CE inhibition.

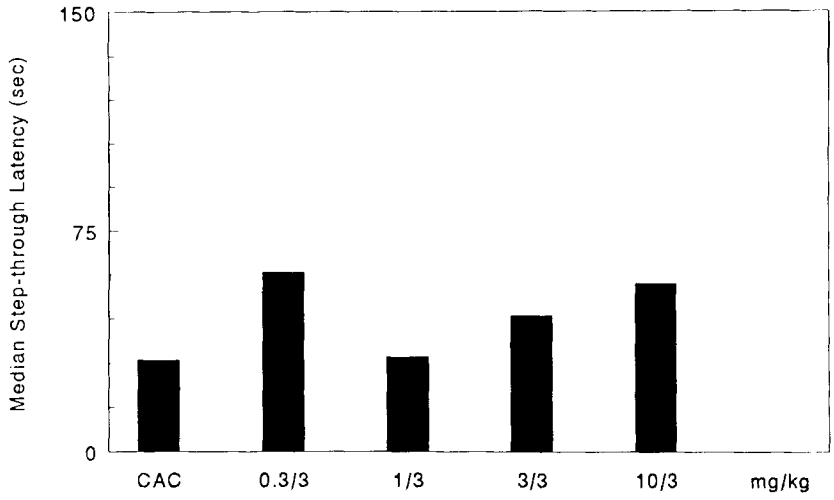

Fig. 7. Effects of the combination of RA-octil (i.p.) and L-NNA (i.p.) on inhibitory avoidance in mice. CAC, control group (received vehicle only). Under each bar, the first number represents the dose of RA-octil; the second represents the dose of L-NNA.

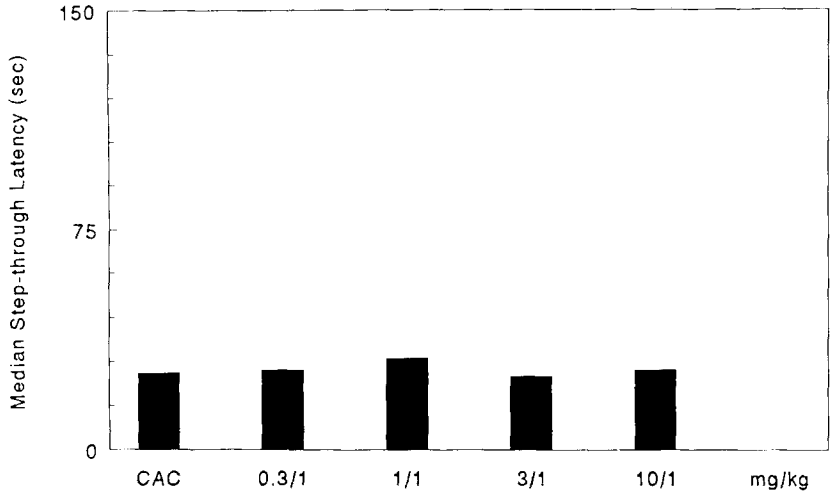

Fig. 8. Effects of the combination of RA-octil (i.p.) and Hoe 140 (s.c.) on inhibitory avoidance in mice. CAC, control group (received vehicle only). Under each bar, the first number represents the dose of RA-octil; the second represents the dose of Hoe 140.

As mentioned in the Introduction, NO played a possible role in LTP, which is the electrophysiological correlate for learning and memory. In an in vitro study on rat hippocampus slices Böhme et al. [1991] showed that L-N^G-nitro-arginine (L-NOARG) blocks LTP induction in a manner that could be reversed stereospecifically by L-arginine, the substrate of NO-synthase. L-N^G-monomethyl-arginine (L-NMMA), another inhibitor of NO-synthase [Knowles et al., 1990] has also recently been shown to block another form of synaptic plasticity in the brain, namely long-term depression in the cerebellum [Shibuki and Okada,

1991]. The same authors also showed that exogenous NO or cGMP could substitute for the stimulation of climbing fibers to cause long-term depression in rat cerebellar slices. Both results showed that NO is essential for the induction of synaptic plasticity in the brain.

Taken together the results show that a prolongation of step-through latencies by ramipril and RA-octil might be gated via endothelial-derived bradykinin. This could be explained by the ineffectiveness of ramipril and RA-octil, respectively, in combination with L-NNA or Hoe 140. It was interesting that both compounds showed similar effects in the central nervous system whereas a clear difference existed in the periphery [Wiemer et al., 1991a]. At this time we do not have a final explanation, and further studies are underway to confirm this possible explanation.

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