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Treatment with dehydroepiandrosterone sulfate increases NMDA receptors in hippocampus and cortex

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Abstract

Our aim was to investigate if the memory-enhancing effects reported for dehydroepiandrosterone sulfate in rodents could be mediated through modulation of NMDA receptors. Using autoradiography we studied the effect of dehydroepiandrosterone sulfate, administered for 5 days (30 mg/kg, i.p. twice a day), on NMDA binding sites labelled with [^{3}H]dizocilpine ([^{3}H]MK801) in rat brain. Dehydroepiandrosterone sulfate treatment significantly increased the [^{3}H]MK801 binding sites in hippocampal areas (field CA1, CA3, dentate gyrus lateral blade and medial blade) and in cortex layer IV as compared to the control group. These results demonstrate for the first time the ability of dehydroepiandrosterone sulfate to increase the number of NMDA binding sites in rat brain, an action that could be of interest for therapeutic application.

Keywords: Dehydroepiandrosterone sulfate; NMDA receptor; Brain; (Rat)

Neurosteroids are present in the central nervous system (CNS) and can be allosteric modulators of neurotransmitter receptors (Vincens et al., 1992). In fact, several metabolites of progesterone such as allopregnanolone and pregnanolone bind to the $GABA_A$ receptor complex and are positive allosteric modulators of this $GABA_A$ receptor complex (Vincens et al., 1992). Other studies have suggested that pregnenolone sulfate could modulate the NMDA receptor, a subtype of glutamate receptor (Wu et al., 1991). These NMDA receptors are known to be involved in long-term potentiation and in learning and memory (Tsien, 2000). Moreover, dehydroepiandrosterone sulfate, another neurosteroid, has been reported to have significant memory-enhancing effects in vivo in mice (Wolkowitz and Reus, 1996). This effect could result from modulation of NMDA receptors. In order to investigate this hypothesis, we studied in rat brain the effects of dehydroepiandrosterone sulfate treatment on NMDA receptors labelled with the specific ligand [^{3}H]dizocilpine ([^{3}H]MK801) (NEN Boston, MA), which reveals all NMDA receptor subtypes.

Fourteen male Wistar rats (230–250 g) (seven animals per group) received an intraperitoneal (i.p.) injection of either the vehicle or the substance to be tested, twice a day at 8 and 19 h for 4 days. The last injection was at 8 h on the fifth day. All the animals were killed 2 h after the last injection. The control group received only the vehicle, dimethyl sulfoxide (DMSO, Aldrich; 0.2 ml/250 g body weight (b.w.)). The treated animals received dehydroepiandrosterone sulfate (Sigma; 30 mg/kg b.w.) dissolved in DMSO (0.2 ml/250 g b.w.).

Brain tissues were processed as already described by Vincens et al. (1995). The autoradiographic technique described by Porter and Greenamyre (1995) was used: mounted brain sections were incubated with 7 nM [^{3}H]MK801 (total binding). Alternate sections were incubated with 10^{-5} M dizocilpine to assess non-specific binding.

Binding was measured as relative optical density (OD) units. Under these experimental conditions, non-specific labelling density averaged less than 5% of the total [^{3}H]MK801 and was subtracted from the OD readings.

The autoradiographic distribution of [^{3}H]MK801 specific binding in midbrain of control rats (Fig. 1A) showed that the highest densities of [^{3}H]MK801 binding sites, expressed as OD (mean ± S.E.M) were found in cortex (layer II–III, OD: 40.54 ± 1.3) and hippocampus (field CA1, CA3, dentate gyrus, lateral blade and medial blade; ODs are: 44.76 ± 1.5; 28 ± 1.7; 37.4 ± 1.4; 36.2 ± 1.08, respectively). Intermediate binding sites occurred in cortex (layer IV, OD: 21.3 ± 1.7; layer V–VI, OD; 19.1 ± 2.3)

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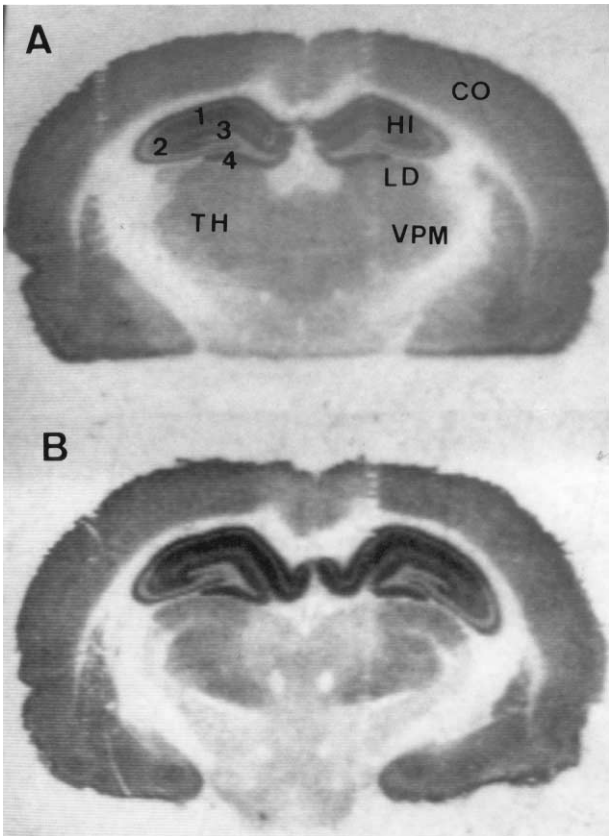

Fig 1. Digitalized image (Biocom, les Ulis, France, RAG-200) of an autoradiographic film showing the density of total [^{3}H]MK801 binding sites in rat midbrain (coronal section at level p29 of the Swanson atlas) control rats (A) and dehydroepiandrosterone sulfate-treated rats (B) (CO: cerebral cortex; HI: hippocampus, 1: field CA1, 2: CA3, 3: dentate gyrus lateral blade, 4: dentate gyrus medial blade; TH: thalamus; LD: lateral dorsal thalamus; VPM: ventral posteromedial nucleus thalamus).

and in thalamus (lateral dorsal thalamus and ventral posteromedial nucleus; ODs: 18.2 ± 2.1 and 17.4 ± 2.3, respectively).

After dehydroepiandrosterone sulfate treatment, the apparent number of [^{3}H]MK801 binding sites was significantly increased in hippocampal areas (field CA1, CA3, dentate gyrus lateral blade and medial blade) and in cortex layer IV by 22%, 18%, 13%, 19% and 14%, respectively (OD: $54.01 \pm 1.58^{**}$; $33.37 \pm 2.1^{**}$; $42.15 \pm 1.2^{**}$; $43.13 \pm 1.7^{**}$; $24.88 \pm 1.8^{*}$) as compared to the control group ($^{*}P < 0.01$, $^{**}P < 0.001$). No significant changes were observed in cortex layers II–III and V–VI and in thalamus (lateral dorsal and ventral posteromedial nucleus) (Fig. 1B).

It is worth noting that this increase was obtained after 5 days of treatment only. A single injection did not induce any modification at 2 h following treatment (data not shown), which does not suggest a change in receptor affinity.

The present data demonstrate for the first time the ability of dehydroepiandrosterone sulfate treatment to increase NMDA binding sites specifically in hippocampus and cortex, structures implicated in memory processes. Since activation of NMDA receptors has been implicated in learning and memory (Tsien, 2000), the improvement of memory by dehydroepiandrosterone sulfate treatment could be the result of an increase of NMDA receptors. These findings are of special interest since NMDA receptors decrease with age in rodents and humans. A decrease was also found in the brain of patients with senile dementia of Alzheimer's type (Ulas et al., 1992). Therefore, a decline in this receptor might underlie age-related deficiencies in learning and memory.

Moreover, a physiological decrease in dehydroepiandrosterone and dehydroepiandrosterone sulfate plasma levels in both men and women has been reported with ageing (Ravaglia et al., 1996).

These findings, together with the fact that during ageing, a physiological decrease of NMDA receptors and dehydroepiandrosterone sulfate plasma levels is observed in humans, make it of great interest to prevent the decrease in NMDA receptors by treatment with dehydroepiandrosterone sulfate, at the time of the decline of dehydroepiandrosterone sulfate plasma levels in both men and women.

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