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Neuroscience Letters 383 (2005) 155–159
Neuroscience Letters
www.elsevier.com/locate/neulet

The effects of haloperidol treatment on the distribution of NK_1 receptor immunoreactive neurons in guinea-pig brain

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Received 17 February 2005; received in revised form 1 April 2005; accepted 2 April 2005

Abstract

Previous studies have observed increased tachykinin NK_1 receptor immunoreactivity (NK_1-IR) in the prefrontal cortex in subjects with schizophrenia. Since the subjects were medicated the possibility of a treatment effect could not be excluded. Thus, the present study was undertaken to determine the effect of chronic treatment with the antipsychotic drug, haloperidol, on the distribution of NK_1-IR neurons in the guinea-pig brain. Guinea pigs were treated each day for 21 days with either haloperidol (1 mg/kg) or vehicle and the brains were then processed for immunohistochemistry using an NK_1 receptor-specific polyclonal antibody. NK_1-IR neurons and fibres were abundant in the forebrain cortex and caudate putamen and more sparsely distributed in a number of other brain regions. The relative density of NK_1-IR neurons was significantly increased in the forebrain cortex, but not in the caudate putamen in guinea pigs treated with haloperidol. This study has shown that haloperidol causes region-specific changes to the density of NK_1-IR neurons. Whether these changes are related to the therapeutic effects or to the side effects of haloperidol in individuals with schizophrenia, remains to be determined.

Keywords: Tachykinins; NK_1 receptor; Haloperidol; Schizophrenia; Guinea pig

The tachykinins are neuropeptides found in the central and peripheral nervous systems that produce marked behavioural changes in experimental animals [7,15]. The tachykinin family includes substance P, neurokinin A and neurokinin B. A new tachykinin, hemokinin-1, was discovered in haemopoietic cells and appears to bind to the NK_1 receptor [21]. The tachykinins exert their effects through activation of three types of transmembrane receptors termed NK_1, NK_2, and NK_3 [12,13]. Substance P, neurokinin A and neurokinin B bind preferentially, but not exclusively to NK_1, NK_2 and NK_3 receptors, respectively [12].

Several studies over the past decade have used various techniques to investigate the cellular localization of the NK_1 receptor in the brain. In a study of the guinea-pig brain, NK_1 receptor-immunoreactive (NK_1-IR) neurons were predominantly observed in the caudate putamen and the superficial layers of the cortex [20]. NK_1-IR neurons were also seen in thalamic and hypothalamic regions, with fewer positive neurons in the mid- and hind-brain regions [20]. In the human brain, NK_1 receptors have been localised to the striatum [2,4], cerebral cortex [4,6,16], locus coeruleus, hippocampus and amygdala [4].

Schizophrenia is a disabling psychiatric disorder with a life-time risk of 1% for the general population. Dopamine and several other neurotransmitter systems in the central nervous system have been implicated in schizophrenia. Although there has been speculation concerning the possible role of the tachykinins in schizophrenia, few studies have been carried out. The first report linking tachykinins and schizophrenia was that by Roberts et al. [14] who found elevated levels of substance P in the hippocampus from subjects with schizophrenia. Other studies have shown increased

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doi:10.1016/j.neulet.2005.04.012

immunoreactivity for substance P in 10 brain regions [19] and increased binding of [I-125]BH-substance P in the caudate nucleus and nucleus accumbens in subjects with schizophrenia [13].

A recent study using an anti-human NK_1 receptor antibody showed a significant increase in the NK_1-IR in the prefrontal cortex in schizophrenia compared to control subjects [18]. In addition, the NK_1-IR was distributed across all cortical layers in subjects with a diagnosis of schizophrenia and not restricted to the upper cortical layers as seen in control subjects [18]. The possibility was raised that the neuroleptic medication used in the treatment of schizophrenia might be responsible for the observed increases in NK_1-IR. Indeed, studies in rat brain have shown that treatment with typical and atypical antipsychotic drugs induces region-specific changes in the levels of the tachykinins [1,5,8–11]. However, only one study has investigated the effects of antipsychotic drugs on the distribution of the tachykinin receptors. This study in the rat brain assessed the binding of [I-125]BH-substance P (representing mostly NK_1 receptors) in the striatum and substantia nigra after treatment for 10 days with either haloperidol or clozapine and observed no changes in response to these drug treatments [5].

The aim of the present study was to determine if treatment with the typical antipsychotic drug, haloperidol, affects the distribution of NK_1 receptors in guinea-pig brain. Guinea pigs were chosen since their tachykinin receptors are closer in pharmacology and sequence homology to the human type, than are the rat or mouse types.

Adult tricolour guinea pigs of either sex weighing 300–500 g were supplied by the Central Animal House of the University of Newcastle. Ethics approval for the study was obtained from the Animal Care and Ethics Committee of the University of Newcastle (#7050501). Every effort was made to minimize pain and discomfort during experimentation that was carried out according to the Australian code of practice for the care and use of animals for scientific purposes 7th Edition 2004. The guinea pigs were housed in pairs in the animal rooms for 1 week prior to beginning experiments. During this time and for the duration of the experiments, the animals were weighed daily. The animal rooms were maintained at constant room temperature (23 °C) and on a 12 h/12 h light/dark cycle. Standard guinea-pig pellets and water were made available ad libitum and Vitamin C added to the drinking water daily.

For all experiments, guinea pigs were acclimatised to the locomotor cages for 30 min prior to injection of drugs. To determine the dose of haloperidol (Serenace®, Sigma Pharmaceuticals, Vic., Australia) that effectively blocked the D_2 receptor in guinea pigs, separate groups of four guinea pigs were injected with haloperidol (0.1, 1.0 or 10 mg/kg, s.c.) or saline (s.c.), 30 min prior to injection of quinpirole hydrochloride (3 mg/kg, s.c) [3]. Quinpirole hydrochloride is a D_2 receptor agonist that induces an increase in locomotor activity. The guinea pigs were then placed in separate activity cages and locomotor activity measured for 60 min. At the end of the experiment, guinea pigs were sacrificed by

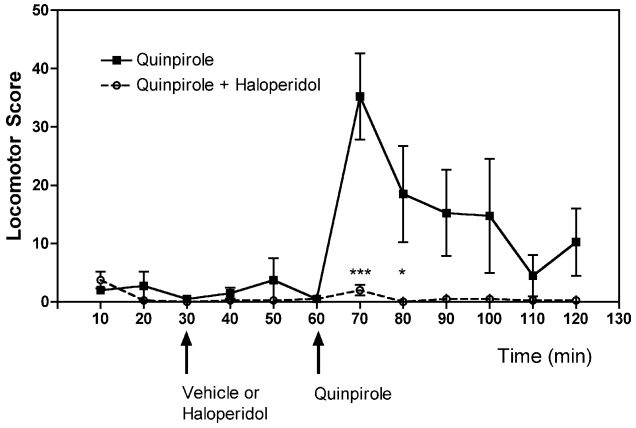

Fig. 1. Graph of the locomotor response in guinea pigs treated with vehicle or haloperidol (1 mg/kg) 30 min prior to treatment with quinpirole hydrochloride (3 mg/kg). Quinpirole hydrochloride induced locomotor activity above resting levels in the guinea pigs which was completely blocked by haloperidol pre-treatment ($^{*}P < 0.05$, $^{***}P < 0.001$ by two-way ANOVA with Bonferroni post-tests).

injection of sodium pentobarbitone (100 mg/kg). Locomotor scores were obtained by adding the total number of times a guinea pig crossed an infrared beam over successive 10 min intervals. Locomotor scores were analysed by two-way analysis of variance with Bonferroni post-tests. In agreement with previous reports [3], treatment of guinea pigs with quinpirole hydrochloride at 3 mg/kg induced a robust locomotor response (Fig. 1). This locomotor response was completely blocked by injection of haloperidol at 1 (Fig. 1) or 10 mg/kg, but not 0.1 mg/kg (data not shown).

To determine the effect of haloperidol on the distribution of NK_1-IR neurons in the brain, separate groups of four guinea pigs were injected, s.c. once a day for 21 days with 1 mg/kg haloperidol or saline. On day 22, guinea pigs were injected with sodium pentobarbitone (100 mg/kg) and perfuse-fixed with 4% paraformaldehyde in phosphate buffer. The brains were removed, post-fixed overnight and placed in 30% sucrose solution in 0.5% paraformaldehyde for cryoprotection.

Brain tissue was sectioned at 50 μm and one in five sections placed in a multi-well tray containing PBS. Floating tissue sections were washed with PBS, followed by 20 min in 0.9% hydrogen peroxide/PBS and 15 min in 0.2% Triton X-100/PBS, then blocked for 30 min in 10% normal donkey serum (NDS)/0.1% sodium azide/PBS. All incubations were at room temperature. Sections were then incubated with rabbit anti-NK_1 receptor antiserum (Novus Biologicals, USA) diluted in 0.1% sodium azide/0.1% NDS/0.2% Triton X-100/PBS for 48h at 4 °C. This antibody has been used to successfully immunostain the NK_1 receptor-positive neurons in guinea-pig brain [20]. Normal rabbit serum was substituted for the primary antibody in negative controls. Sections were washed three times for 15 min in PBS, before incubation in biotinylated donkey anti-rabbit IgG (1:1000 in 0.1% NDS/PBS; Jackson Laboratories, USA), followed by the avidin–biotin horseradish peroxidase complex (ABC; Vectastain Elite kit,