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The AMPA receptor modulator S18986 in the prelimbic cortex enhances acquisition and retention of an odor-reward association

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Abstract

Systemic administration of S18986, a positive allosteric modulator of AMPA receptors, improves cognition. The present study further characterizes the drug's memory-enhancing properties and is the first to investigate its intracerebral effects on learning and memory. The results showed that rats receiving a single dose of S18986 (3µg / site) into the prelimbic cortex, prior to olfactory discrimination acquisition, exhibited significantly shorter latencies and fewer errors to make the correct response, both in the acquisition and two drug-free retention tests. Such findings corroborate the involvement of glutamate receptors in odor-reward learning and confirm the role of the AMPAkine S18986 as a cognitive enhancer.

Keywords: olfactory discrimination, memory facilitation, cognitive enhancer, prefrontal cortex, glutamate, AMPA receptors.

1. Introduction

(S)-2,3-dihydro-[3,4]-cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide (S18986) is a positive allosteric modulator of the glutamatergic AMPA receptors (AMPAR) that has recently been proposed as a novel cognitive enhancer [1]. The beneficial effect of S18986 on cognitive processes has mainly been studied with oral administration, demonstrating positive results in spatial memory [1,4,15], operant-delayed alternation [11], contextual serial discrimination [7], delayed spatial discrimination [21] and object recognition [2,12]. Intraperitoneal administration, which has been studied to a lesser degree, also improved social recognition [1], spatial working memory [22] and counteracted scopolamine-induced impairment in passive avoidance memory [17]. As for its cellular effects, S18986, like other positive AMPAR modulators, would seem to be important for synaptic plasticity underlying learning and memory as it increases long-term potentiation (LTP) induction and maintenance in the hippocampus [1]. Moreover, the drug enhances the expression of cortical and hippocampal brain-derived neurotrophic factor (BDNF) (for references see [1,14]), which regulates neuronal plasticity. In addition to its effects on LTP and BDNF expression, the cognitive-enhancing properties of S18986 may reside in its ability to control the release of several neurotransmitters, as it enhances acetylcholine, noradrenaline and dopamine in the hippocampus and/or frontal cortex [13,17]. It has been demonstrated, by means of in situ brain perfusion, that brain areas on which S18986 may act are the hippocampus and frontal cortex [6].

Nevertheless, a better understanding of S18986 actions continues to be an important issue as knowledge of its intracerebral targets is relatively unknown. In this context, the present study aimed to investigate whether intracerebral S18986 would produce a promnesiant effect, similarly to what has been found in the case of systemic administration. A learning model sensitive to manipulations of the glutamatergic receptors is the odor discrimination task (ODT), which entails an odor-reward association, is rapidly learned and does not involve fear or acute stress [19]. A frontal cortical area that has been specifically linked to ODT is the prelimbic

cortex (PLC) [19,20], which receives strong projections from the olfactory bulbs [23] and is rich in AMPAR and NMDA receptors (NMDAR). Furthermore, previous studies in the ODT demonstrated that infusions into the PLC of d-cycloserine (DCS), a partial agonist of the NMDAR, enhanced relearning [25] and reduced brain damage-induced deficits [24]. Thus, using the ODT, the present research further explores the role of AMPAR modulation in cognition by evaluating the effects of pre-training intra-PLC administration of S18986 on acquisition and retention.

2. Material and Methods

2.1. Subjects

Twenty-eight male Wistar rats with an initial mean weight of 408.09 g (SD = 34.54) were used. All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (86/609/ECC) and Generalitat de Catalunya authorization (DOGC 2450 7/8/1997, DARP protocol number 5959). For detailed descriptions of materials and methods see [8] and [25]. The rats were submitted to a food restriction schedule for five days (12 g/day, to maintain their body weight at 85% of their free-feeding weight), after which they performed three habituation sessions to become familiarized with the reinforcement and the training box. The rats were then returned to *ad libitum* conditions and surgery was carried out.

2.2. Surgery and microinfusion

The stereotaxic coordinates for the PLC were: AP: +3.5mm from bregma, ML: ±0.6mm from midline, and DV: −2.9mm from cranium surface [16]. The day after the 4-day recovery period, the rats were again food-restricted (12 g/day) for 2-3 days and submitted to an additional habituation session. One day after the fourth habituation, ODT acquisition training was carried out. Infusions of S18986 (3µg/site) or vehicle (dimethyl sulfoxide, DMSO) were administered (0.5µl / hemisphere for 2 minutes) ten minutes before ODT acquisition (parameters chosen according to a pilot study).

2.3. Behavior

2.3.1. ODT

ODT acquisition involved a single four-trial session in the training box (60 cm×60 cm×40 cm), containing three sponges (8.5cm×6.5 cm×6.5 cm) located in any three of the four corners of the box. Chocolate rice cereal was placed into a hole in the target sponge so that the rat was obliged to poke its muzzle (nose-poke) to obtain the reinforcement, which was associated with the same odor across trials. The location of the odorized sponges (0.2 ml vanilla, 0.6 ml orange and 0.3 ml anise; Vahiné, Spain) within the box was changed for each trial according to a previously determined protocol. The inter-trial interval was 1 min and there was a 3 min limit, in each trial, for the rat to find and consume the reinforcement. A behavioral criterion eliminated rats failing to nose-poke within a 3-min period by the fourth trial of the acquisition session from the main analyses. Latency before a correct response (nose poke into the reinforced sponge) and number of errors were scored. Two different kinds of errors were combined: errors of commission (nose-poke into incorrect sponges) and omissions (failure to nose-poke after sniffing the sponge containing the target odor). Motor behavior was analyzed using SMART (SMART v3.0, Panlab, Spain) video tracking system, recording the mean speed and distance covered to reach the sponge with the target odor. Twenty-four and forty-eight hours after acquisition, the rats were examined in drug-free tests using the same procedure as during training, with the exception that the first test trial was not reinforced, to provide a direct measure of memory of the previous training [20].

2.3.2. Olfactory perception test

To rule out olfactory alterations due to S18986 infusion, an additional olfactory perception test was conducted at the end of the experiment [5]. The test was carried out in clean rat cages (50 x 22 x 14 cm) and a piece of cookie (Brambly hedge, Denmark) was buried in one corner of the cage. Ten minutes after infusion with either S18986 or VEH, the rats were placed in the cage and the latency in finding the buried cookie and commence eating was timed. Upon completion of the behavioral tests, the rats were subjected to histological verification of cannulae placements following procedures explained elsewhere [8].

2.4. Data analysis

Data (latencies and errors) were submitted to a mixed analysis of variance using repeated measures (ANOVA; IBM SPSS Statistics v20) in which the between-factor was Group (S18986 and VEH) and the within-factor was Session. The Session factor consisted of 3 levels (the average scores of the 4 trials in each session): acquisition, 24-hour test and 48-hour test. Ancillary analyses were performed to control several variables that could have influenced the main results. Firstly, changes in motivation towards the reinforcement due to the surgical procedure were analyzed (ANOVA) by comparing the mean latencies to eat the cereal in habituations three and four. Secondly, the possible effects of S18986 on olfactory perception were analyzed by means of ANOVA, considering Group (S18986 and VEH) as the independent variable and Latency in finding the buried cookie as the dependent variable. Finally, an ANOVA analysis of motor behavior was performed in a subset of animals (S18986, n=6; VEH, n=7), considering the measures Distance covered and mean Speed to reach the target sponge. P-values less than 0.05 were considered to be significant.

3. Results

3.1. Histology

For the final sample we only considered rats with their microinjector tips in the PLC within the area delimited by the anterior cingulate and infralimbic cortices and in which no tissue damage, due to the rate or volume of the infusions, was detected (Figure 1). Incorrectly implanted cannulae (n=2), the existence of problems during surgery (n = 2) and failure to fulfill the behavioral criterion (n=5) were considered grounds for exclusion. Thus, the final sample was made up of 19 subjects distributed into VEH (n = 9) and S18986 (n = 10) groups.

3.2. Behavior

As depicted in Figure 2, S18986 improved the acquisition and both retention tests as it reduced latency and number of errors. As for latencies (Fig. 2A), the ANOVA analysis showed that the Group ($F_{(1,17)}$= 5.721; P= 0.029) and Session ($F_{(2,34)}$= 8.077; P= 0.001) factors were statistically significant, which was not the case for the interaction Group x Session ($F_{(2,34)}$=0.832; P=0.444). The analysis of total errors showed a similar pattern of results (Fig. 2B), as Group ($F_{(1,17)}$= 8.116; P= 0.010) and Session ($F_{(2,34)}$= 3.554; P= 0.040) proved to be

statistically significant, which was not the case for Group x Session ($F_{(2,34)}$= 0.775; P= 0.469). The test comparing the latencies of habituations three and four showed that neither Group ($F_{(1,17)}$= 5.721; P= 0.291), Session ($F_{(1,17)}$= 3.961; P= 0.063) or Group x Session ($F_{(1,17)}$= 1.216; P= 0.286) were statistically significant. There were no deficits in olfactory sensitivity as no statistically significant between-group differences for the latency in finding a buried cookie were found ($F_{(1,17)}$= 1.388; P= 0.255). There were no alterations in motor behavior (Table 1) as no statistically significant between-group differences were found in mean Speed ($F_{(1,11)}$= 0.803; P= 0.389) or Distance ($F_{(1,11)}$= 2.461; P= 0.145) to reach the target sponge.

4. Discussion

The present study showed that S18986 improved an olfactory discrimination task in that rats receiving a single infusion in the PLC prior to acquisition displayed significantly shorter latencies and fewer errors than vehicle-infused rats, in all the ODT sessions (acquisition, 24h and 48h retention tests). Such findings cannot be attributed to alterations in olfactory perception or locomotor activity since S18986 infusions did not have any effect on the latency in finding a buried sweet-smelling cookie or the speed and distance covered to reach the rewarded odor. A state-dependent learning as a consequence of the prior-to-acquisition infusions can also be discarded as S18986 rats exhibited a better performance than vehicle rats in the drug-free memory tests. The current findings corroborate that the glutamatergic transmission in the PLC is essential for such discrimination learning based on odorous stimuli. A previous study demonstrated that rats infused in the PLC with the cognitive enhancer DCS (a partial agonist of the NMDA receptor glycine site) exhibited a significant enhancement of ODT performance, especially in terms of relearning [25]. Furthermore, it has recently been shown that DCS in the PLC attenuated parafascicular lesion-induced deficits on ODT acquisition and retention [24]. The present results are, therefore, the first to demonstrate that the AMPAR potentiator S18986 infused directly into the brain, specifically in the PLC, enhances learning and memory in adult rats and support the role of the drug as a cognitive enhancer.

Our findings cannot be directly compared to previous results obtained with S18986 administration since, to our knowledge, there are no existing studies aimed at evaluating the effects of intracerebral infusions on behavioral tasks. However, facilitative effects on a number of learning paradigms have consistently been described with systemic (oral or intraperitoneal) administrations. Although memory-enhancing effects appeared to be stronger in episodic-like and spatial memory tasks in middle-aged animals (for references see [1]), they have been demonstrated in tasks exploring different types of memory, including procedural [17], working and relational/declarative [15], episodic contextual memory [7], in young [12], adult and aged rodents [15,17]. Therefore, our results, together with others, show that the beneficial effects of a S18986 treatment may also be extended to several kinds of memory paradigms in animals of different ages.

In terms of its action mechanisms, S18986 does not interact with the glutamate binding site, but rather with the receptor at an allosteric site and augments the receptors' function [3]. This boosts Ca^{2+} influx, increasing amplitude and/or duration of excitatory postsynaptic potentials and thus enhancing synaptic responses. Moreover, AMPAR activation exerts an indirect regulation of NMDAR activation (involved in synaptic plasticity mechanisms), which may prevent the overactivation of such receptors and reduce the excitotoxic effects related to the neuronal degeneration observed during ageing and age-related disorders [10]. Indeed, neuroprotective and neurotrophic properties of S18986 have been described [9], and it has been suggested that they may be involved in its prevention of age-related cognitive deficits. In accordance with this suggestion, a recent study has shown that systemic chronic administration of S18986 at low doses in aged rats increased the expression of BDNF and also enhanced spatial memory [4]. In addition to their effects on LTP and neurotrophic factors, memory-enhancing effects of AMPA allosteric modulators may lie in their capacity to modulate several neurotransmitter systems. Thus, S18986 enhances acetylcholine in the hippocampus of freely moving rats, noradrenaline in both hippocampal and frontal cortex slices, and dopamine in frontal cortex slices [13,17]. Such neurotransmitter release in the frontal cortex may underlie

memory improvement as the activation of closely related brain regions, particularly in the frontal cortex (PLC, infralimbic, orbital) and the amygdala, has been described specifically for ODT [19]. Interestingly, the involvement of noradrenaline in the PLC has been demonstrated in ODT consolidation [18]. Therefore, all the above data suggest that the improving effects of S18986 on learning and memory observed in the present experiment may be associated with the enhancement of synaptic plasticity mechanisms, neurotrophic factors and/or neurotransmitter release within the PLC neuronal networks related to ODT.

5. Conclusions

In summary, the present study shows that infusions of S18986, a positive allosteric modulator of the AMPAR, in the medial prefrontal cortex, specifically the PLC, enhanced learning and memory of an odor-reward task. Although direct infusions in the brain represent a precise way of exploring its potential benefits and effects on cognitive processes, our study is the first to explore the effects of intracerebral S18986. Taken as a whole, the cognitive-enhancing properties of S18986 demonstrated in the present rodent model of olfactory memory together with previous reports would indicate that S18986 may be useful in the treatment of memory disorders associated with age-related cognitive dysfunctions and neuropsychiatric conditions. We consider that additional research on the effects of S18986 infusions in other brain regions, such as the hippocampus, using different infusion protocols and learning paradigms (e.g. relational), would be necessary to further explore the neural mechanisms involved in its facilitative effect on memory.

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Table Legend

Table 1. Mean speed (cm/sec) and mean distance (cm) to reach the target odor in each ODT session for each group.

Figure legends

Figure 1. (A) Left: Photomicrograph (2× magnification) of cresyl violet staining at the level of PLC showing the cannula track and the microinjector tip of a representative subject; Right: stereotaxic plate providing PLC location and coordinates (AP, 4.2 mm anterior to bregma). (B) Location of injectors within the PLC. Schematic representation of the brain at two rostro-caudal levels (4.2 and 4.7 mm anterior to bregma). Each asterisk represents one subject.

Figure 2. (A) Mean latency (± SEM) and (B) mean number of errors (± SEM) in each ODT session for each group. The injection of DMSO (VEH) and S18986 was administered 10 minutes before the acquisition session.