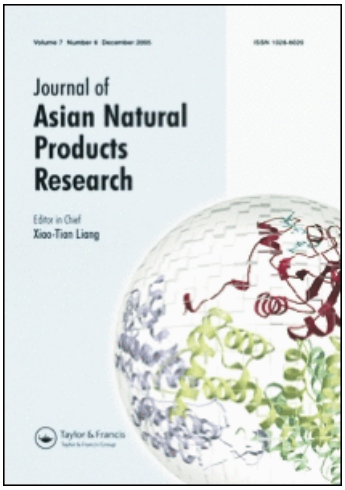

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Two forms of long-term potentiation induced by different compounds

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Two forms of long-term potentiation induced by different compounds

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The purpose of this study was to investigate different forms of long-term potentiation (LTP) induced by different compounds. As shown in results, the influx of calcium via voltage-dependent calcium channels (VDCCs) was responsible for (−)-clausenamide-induced LTP in hippocampus, while Rg1 induced LTP is mediated by the entry of calcium via *N*-methyl-D-aspartate glutamate (NMDA) receptor. The findings in the present study provide pharmacological basis for the nootropic mechanisms of these two compounds.

Keywords: Clausenamide; Rg1; Long-term potentiation; Voltage-dependent calcium channels (VDCCs); *N*-methyl-D-aspartate glutamate receptor (NMDAR)

1. Introduction

Long-term potentiation (LTP) is the most prominent model for molecular and cellular mechanisms of learning and memory [1]. In the CA1 region of the hippocampus, in which LTP has been studied most extensively, two main forms of LTP have been distinguished. One is depended on the activation of NMDA receptor and which can be blocked by APV (2-amino-5-phosphonovaleric acid), a specific antagonist of the NMDA glutamate receptor; the other is mediated by entry of calcium via VDCC and the antagonist of L-type VDCC nimodipine could inhibit it [2,3].

Clausenamide, with partial chemical structure similar to the pharmacophore of piracetam, a nootropic drug developed in Europe (figure 1), was isolated from the aqueous extract of *Rutoceae Clausena lansium* (*lour*) *Skeels* leaves, and Ginsenoside Rg1, a purified ingredient from the root of *Panax ginseng* C.A. Meyer (Araliaceae), is a well-known and popular herbal medicine used worldwide. In our previous studies, we found that these two compounds could

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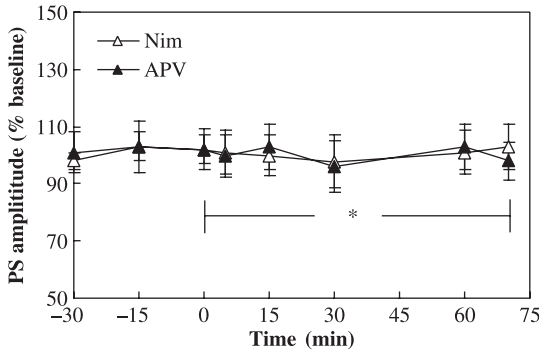

Figure 1. Effects of NMDA receptor inhibitor APV and VDCC inhibitor nimodipine on baseline of population spike amplitude in dentate gyrus of hippocampus in anaesthetised rats. The APV and nimodipine did not affect the baseline of PS amplitude significantly over the duration of the experiments. Each point represents as mean ± SD. $*p > 0.05$ vs. baseline level, $n = 6$ in each group.

facilitate the induction of LTP and enhance the amplitude of population spike in high frequency stimulation induced LTP. The rise in calcium concentration mediated by activation of the NMDAR or VDCCs contribute to formation of LTP. To address different forms of LTP induced by Clausenamide and Rg1 is anticipated in the present study.

2. Results and discussion

2.1 Effect of APV or nimodipine on basic synaptic transmission in anaesthetised rats

Population spike (PS) was elicited at the controlled test stimulation and maintained until the baseline has been stable, and the average amplitude of PS prior to drug injection was defined as 100%. After intracerebroventricular injection (i.c.v.) of 4 nmol nimodipine, an L-type VDCCs blocker, or 5 nmol APV, a specific NMDAR blocker, PS amplitude was recorded for 60 min to determine the effects of agents on the basic synaptic transmission. Results are shown in figure 1. In the APV group, PS amplitudes at 5, 15, 30 and 60 min were respectively 99.7 ± 7.3%, 103 ± 8%, 96 ± 9% and 103 ± 7.8% ($p > 0.05$ vs. baseline, $n = 6$). The corresponding values of nimodipine group were 101 ± 7.6%, 100 ± 7%, 97.8 ± 9.4% and 101 ± 7.6% ($p > 0.05$ vs. baseline, $n = 6$) respectively. It indicated that APV and nimodipine has no effect on basic synaptic transmission.

2.2 Effect of APV or nimodipine on LTP induced by clausenamide in anaesthetised rats

LTP was considered to be successfully induced if the PS amplitude increased to over 130% of its baseline level and this increase lasted for more than 40 min. In these experiments, single administration of (−)-clausenamide could obviously increase PS amplitude and induce the formation of LTP. The percent of PS amplitude was 121 ± 13%, 150 ± 11%, 183 ± 30% at 15, 30, 60 min, respectively (see figure 2). When given nimodipine or APV 10 min prior to (−)-clausenamide administration, (−)-clausenamide-induced LTP was inhibited by nimodipine, the PS amplitude was 98.4 ± 16.7%, 100.8 ± 9.8% at 30 and 60 min; whereas APV had no inhibitory effect on (−)-clausenamide-induced LTP, and the PS amplitude was 163.6 ± 9.8%, 164.6 ± 10.2% at 30 and 60 min, respectively.

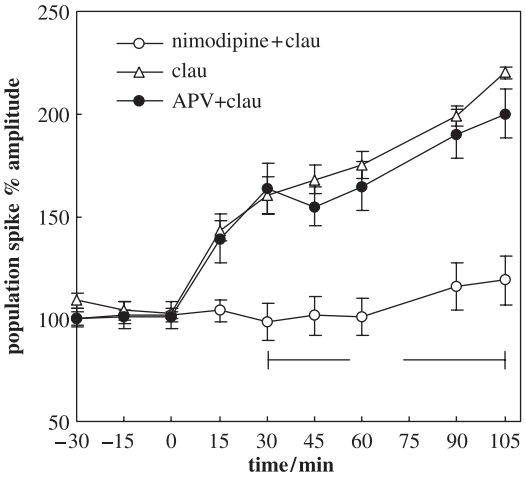

Figure 2. The effect of (−)-clausenamide on synaptic transmission in presence of nimodipine and APV. Nimodipine (4 nmol/L) or APV (5 nmol/L) was delivered i.c.v. 10 min before (−)-clausenamide administration. Each point represents mean ± SD. $^{**}p < 0.01$ vs. (−)-clausenamide group ($n = 5$ for each group).

2.3 Effect of APV or nimodipine on LTP induced by Rg1 in anaesthetised rats

Single i.c.v. administration of 100 nmol/L Rg1 was also able to induce the induction of LTP, while its PS amplitude was 126.8 ± 16.8%, 165.8 ± 18.2%, 172.8 ± 12.8% at 10, 30 and 60 min. Pretreatment of APV (40 nmol, i.c.v.) inhibited LTP induced by Rg1 ($n = 5$, $P < 0.01$). As shown in figure 3, the percent of PS amplitude was 114.6 ± 9.9%, 125.3 ± 10% and 127.6 ± 11.6% at 15, 30 and 60 min, respectively, while 4 nmol nimodipine i.c.v. did not affect Rg1 on induction of LTP. The PS amplitude was 125.6 ± 12.6%, 163 ± 16% and 170.3 ± 15.6% at the same time points.

(−)-Clausenamide and Rg1 have a potential nootropic effect, both of them being reported to improve cognitive deficiency in memory-impaired animal models [4,5]. In the present study, we found that LTP induced by (−)-clausenamide and Rg1 was different. Hippocampal LTP is thought to reflect a cellular mechanism involved in learning

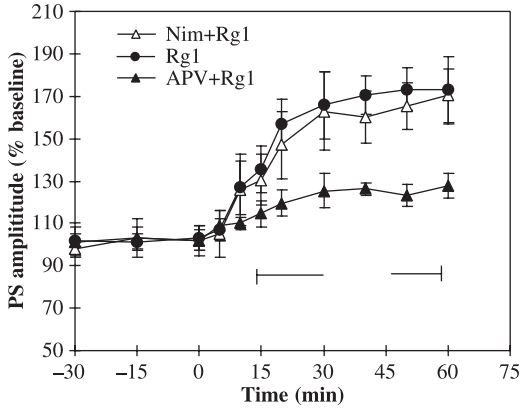

Figure 3. Effects of APV and nimodipine on Rg1-induced LTP in dentate gyrus of hippocampus in anaesthetised rats. APV (40 nmol/L) and nimodipine (4 nmol/L) was injected i.c.v. 10 min before Rg1 administration. All data are presented as mean ± SD of five observations. $^{**}p < 0.01$ vs. Rg1 group.

and memory, therefore these findings may contribute to the nootropic mechanisms of these two compounds.

Associative LTP requires calcium entry into postsynaptic cells, which triggers a cascade of intracellular processes and, if sufficiently strong, ultimately leads to gene expression and protein synthesis [6,7]. Our data showed that both clausenamide and Rg1 mediate calcium influx through VDCCs and NMDARs individually, and then generate two different forms of LTP. While the different forms of LTP appear to reflect the activation of different cellular mechanisms. Cavus and Teyler [8] found that VDCC LTP was blocked by the application of tyrosine kinase inhibitors, whereas NMDA LTP was unaffected. Conversely, serine–threonine kinase inhibitors have no effect on VDCC LTP but block NMDA LTP completely. Therefore, these two forms of LTP have different signal transduction pathways, which resulted in differential regulation of synaptic function and gene expression.

Taken together, the present findings suggest that clausenamide and Rg1 mediated different sources of calcium and induced different forms of LTP, and consequently make unique contributions to memory formation.

3. Experimental

3.1 Animals

Male Sprague–Dawley rats (200 ± 20 g) in this study were provided by the Experimental Animal Center of Chinese Academy of Medical Sciences.

3.2 Electrophysiology

Surgery and electrophysiological recordings were conducted as described previously [9]. Briefly, animals were anaesthetised with urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. Burr holes were drilled in the skull for the drug injection and insertion of electrodes. The cannula for drug delivery was lowered into the lateral cerebral ventricle (0.8 mm from the bregma, 1.8 mm from the midline, 3.0–3.5 mm from the dura). A bipolar stimulating electrode and a unipolar recording electrode were placed in the right perforant path (PP) (7.5 mm from the bregma, 4.2 mm from the midline, 3.0 mm from the dura) and the dorsal cell body region of the ipsilateral dentate gyrus (3.8 mm from the bregma, 2.5 mm from the midline, 3.0 mm from the dura). The PS amplitude was employed as an indication of the excitation level of the granular cell population in the dentate gyrus. A single current pulse was delivered to the perforated path every 30 s; the intensity of stimulation was adjusted to evoke 50% of maximal population spike amplitude (figure 4).

3.3 Drugs and chemicals

Clausenamide (figure 5) was chemically synthesised at the Department of Medicinal Chemistry, IMM, PUMC & CA-MS. Chemical synthesis was described in Juntian Zhang's US patent (No. 6,787,564, assignee: IMM). Rg1 was provided as a present from Bethune Medical University. These two compounds had a purity more than or equal to 98.5% by weight. All other chemicals were purchased from Sigma (St. Louis, MO). In this experiment, clausenamide was dissolved initially in DMSO to make a stock solution of 0.5 mol/L.

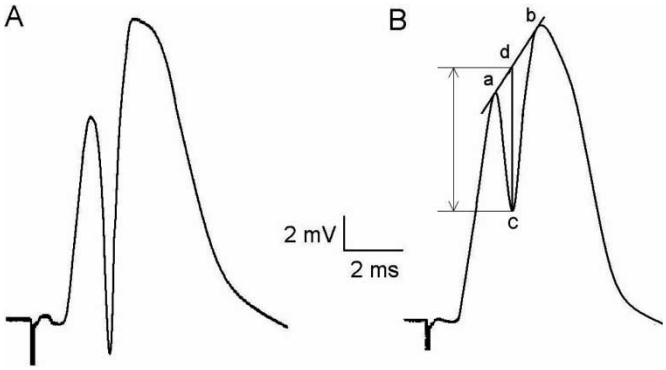

Figure 4. Determination of stimulation intensity for experiments and measurement of population spike (PS) amplitude. (A) Maximal PS amplitude was reached by increasing stimulation intensity. (B) The stimulation intensity was reduced to the desired level for experiments when the population spike amplitude decreased to about 50% of its maximum; the voltage difference between point c and d was measured as the PS amplitude.

All drugs were diluted to their desired concentration with 0.9% NaCl solution (saline) on the day of the experiment. A corresponding dose of DMSO in saline was used as vehicle control for clausenamide and Rg1. All drugs and vehicle were delivered by cerebral ventricular microinjection (i.c.v., 5 μl) via a cannula implanted in the lateral cerebral ventricle after measurement of the baseline for 30 min in the ipsilateral dentate gyrus. Drug doses were calculated on the basis that these drugs would theoretically achieve the brain concentration required, assuming the brain volume to be approximately 2 ml [10]. For example, for an estimated brain concentration of clausenamide at 2.0 μmol/L, 5 μl of clausenamide 800 μmol/L was injected.

3.4 Statistical methods

For each experiment, data are presented as mean ± standard deviation. Statistical differences between the drug and the vehicle treated group were compared by using the Student's *t*-test (two-tailed) and a value of $p < 0.05$ was considered statistically significant.

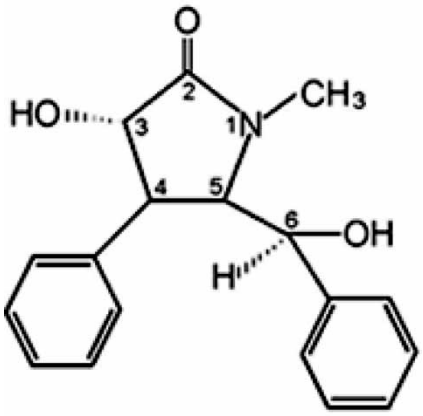

Figure 5. Chemical structure of (−)-clausenamide.

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