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BRIEF COMMUNICATION

Effects of Brain Tissue Hydrolysate on Synaptic Transmission in the Hippocampus

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BASKYS, A. AND J. M. WOJTOWICZ. *Effects of brain tissue hydrolysate on synaptic transmission in the hippocampus.* PHARMACOL BIOCHEM BEHAV **49**(4) 1105–1107, 1994.—A mix of peptides and amino acids obtained from porcine brain tissue (Cerebrolysin) has been shown to affect passive avoidance behavior in neonatal rats. To identify the active components and mechanisms of action, Cerebrolysin effects were studied in in vitro hippocampal slices. Cerebrolysin induced dose-dependent suppression followed by a small rebound increase of synaptic responses in the CA1 but not dentate gyrus neurons. These actions may be due to peptides present in Cerebrolysin and may contribute to its reported behavioural effects.

Cerebrolysin Excitatory synaptic transmission Peptides Hippocampal slices Rat

IT has been reported that low molecular weight tissue-derived endogenous factors can antagonize glutamate toxicity and improve neuronal survival (5,12,16). The commercially available porcine brain hydrolysate, Cerebrolysin (CB; Cerebrolysin™, EBEWE Pharmaceuticals, Austria), has showed mild nerve growth factor-like activity (1), affected passive avoidance behavior in neonatal rats (9), and reportedly improved recent memory and orientation in patients suffering from mild to moderate forms of dementia (8). CB is a mixture of amino acids and small peptides (MW < 10 kDa) (1,9) prepared by standardized enzymatic breakdown of lipid-free pig brain proteins (9) and dissolved in 0.6% saline. It may contain a low molecular weight factor or factors, perhaps of peptide nature, which could cause the reported effects by interacting with synaptic function. As a first step in characterizing these factors, we undertook to examine effects of CB solution on excitatory synaptic transmission in the hippocampus.

All experiments were performed on in vitro hippocampal slices prepared from 15–30-day-old Wistar rats of both sexes by standard dissection techniques. Animals were anesthetized with halothane and decapitated with guillotine. Hippocampal slices (400 μm) were incubated for 1 h in the artificial cerebrospinal fluid (ACSF) and then transferred, one at a time, into the immersion-type recording chamber where ACSF warmed to 30°C was perfused at a rate of 1.5–2.0 ml/min. The ACSF composition was (in mmol): NaCl 124, KCl 3, $CaCl_2$ 2, $MgCl_2$ 2, NaH_2PO_4; 1.25, $NaHCO_3$ 26, glucose 10, and pH 7.3–7.5, when aerated with 95% O_2–5% CO_2. Field excitatory postsynaptic potentials (field e.p.s.p.s), occurring in response to Schaffer collateral/commissural afferent stimulation at 0.1 Hz, were recorded in stratum radiatum of the CA1 area with an ACSF-filled electrode (3–6 MΩ). The initial negative slope of the field e.p.s.p. was taken as a measure of synaptic excitation. Similar recordings were made from dentate gyrus and CA3 areas with stimuli delivered respectively to the perforant path or mossy fiber areas. In all experiments, control recording was continued for at least 20 min to ascertain that the baseline response was stable. Compounds under study were added to the perfusate for 10 min, and recordings were continued for another 30–60 min. Data analysis consisted of comparison of the average change in the experimental group of slices with the mean value obtained by averaging response amplitudes in the 20-min period prior to the CB application. CB effects were compared with those of a synthetic amino acid solution in 0.6% saline (SAAS) (in its composition closely corresponding to the free amino acid composition of CB) and calf blood hemodialysate (Solcoseryl, Solco AG, Basle (6). All solutions were obtained from EBEWE Pharmaceuticals, Austria. Student's two-tailed *t*-test was used to estimate the significance level and results were considered significant if $p < 0.05$. Only those experiments were analyzed where a complete washout of the drug effect occurred. To avoid experi-

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menter bias, the identity of a particular compound was not known during the data collection period.

Results from 31 experiments are summarized in Fig. 1. When measured 5–10 min after the CB application, field e.p.s.p.s were reduced to 26.98% (SEM 3.9, $n = 9$) of the control value. The effect was dose dependent within the range of 2 to 10 μl/ml and could not be abolished by 10 μmol of a selective $GABA_A$ antagonist bicuculline. Application of CB (10 μl/ml) on CA3 neurons in two experiments similarly depressed field e.p.s.p.s (not shown). Depression was significantly weaker in the dentate gyrus area (to 92.6% of the control value, SEM 0.79, $n = 3$).

In five experiments on CA1 neurons, where synaptic responses were followed for 45–50 min after the wash-out of CB, there was a statistically significant increase in the e.p.s.p. amplitude to an average 117.9% (SEM 10.1, $p < 0.05$) of the control value. In the dentate gyrus area, the rebound enhancement at 30–40-min interval was 108.5% (SEM 6.2, $n = 3$, $p > 0.05$) of the control level. Addition of 10 μl/ml of a calf blood hemodialysate solcoseryl (6) to the ACSF did not have any consistent and statistically significant effects on field e.p.s.p.s in the CA1 area (93.6%, SEM 10.7%, $n = 3$, $p > 0.05$, Fig. 1). SAAS was applied in eight experiments, which resulted in reduction of synaptic responses in the CA1 area by an average 40% without the accompanying rebound increase in the field e.p.s.p. amplitude.

These experiments show that the most pronounced, consistent and dose-dependent effect of CB was suppression of synaptic transmission in the CA1 area of the hippocampus. This was followed by a small, delayed enhancement of responses. The absence of responses in the dentate gyrus suggests that there was a certain degree of specificity in the CB action. The suppression could be due to changes in the membrane potential or input resistance, blockade of postsynaptic glutamate receptors or decrease in transmitter release. $GABA_A$ antagonist bicuculline did not block the suppression, indicating that

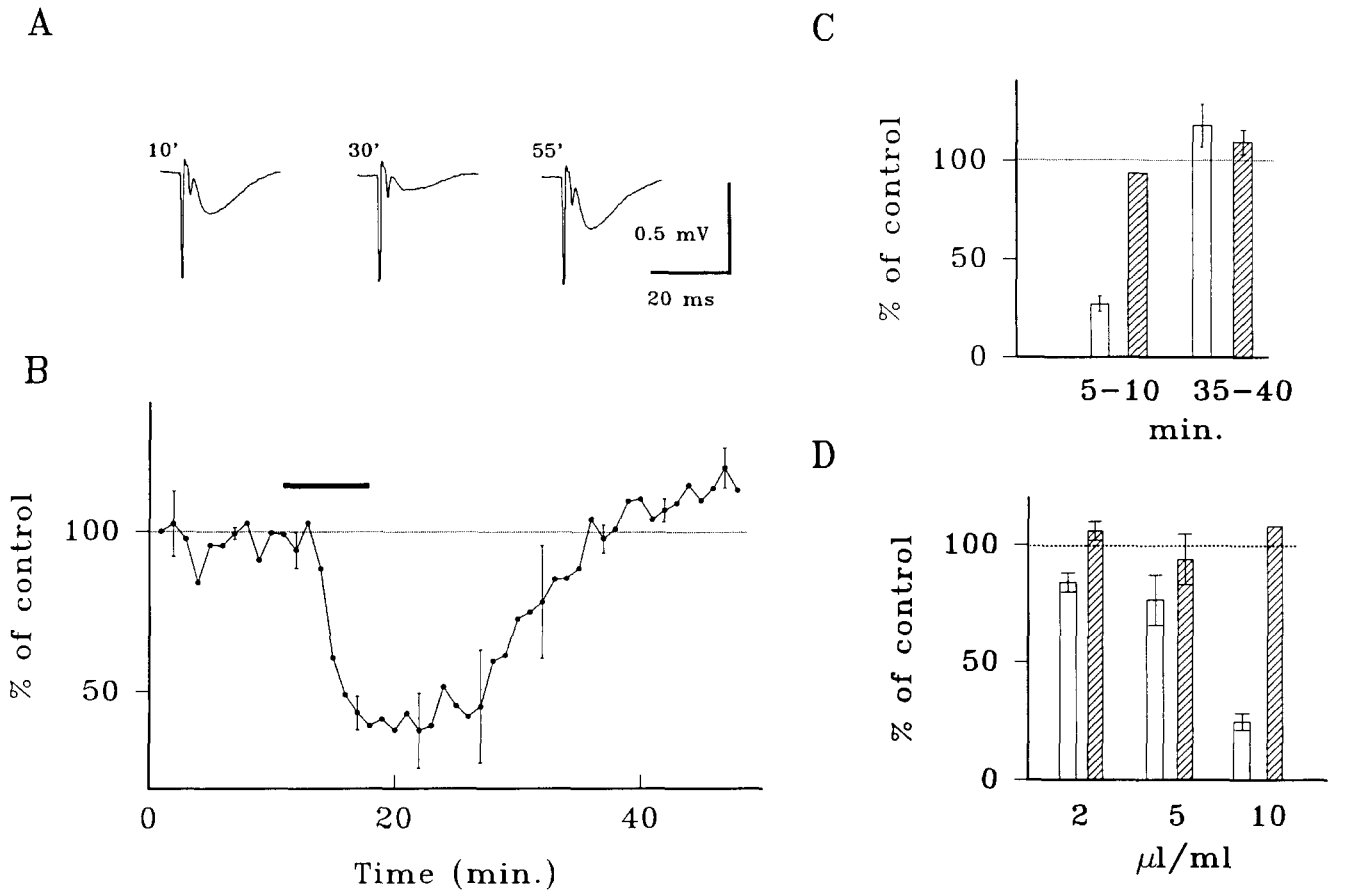

FIG. 1. Cerebrolysin effects on synaptic transmission in the hippocampus. (A) Samples of averaged ($n = 6$) field e.p.s.p.s evoked by stimulation of Schaffer collaterals before, during, and after Cerebrolysin (10 μl/ml) application. Negativity is downward. The large negative deflection marks the stimulation artefact. Numbers indicate sampling time relative to the onset of the experiment. (B) Summary graph showing time course of five experiments where Cerebrolysin (10 μl/ml) was applied for 10 min (bar). Each dot represent the average slope of the field e.p.s.p. Vertical lines, SEM. (C) Average suppression and enhancement of synaptic responses by Cerebrolysin in CA1 (open columns, $n = 9, 5$) and dentate gyrus areas (hatched columns, $n = 3, 3$), in percent to the control level (horizontal dotted line). The control value was obtained by averaging responses within 10 min time interval prior to the Cerebrolysin application. Responses were measured at times indicated below in relation to the onset of Cerebrolysin application. Vertical lines represent SEM. Data were combined from experiments with 10 μl/ml Cerebrolysin. (D) Dose-dependent suppression of field e.p.s.p.s in the CA1 area by Cerebrolysin (open columns, $n = 9, 3, 3$). Hatched columns ($n = 3, 3, 1$), Solcoseryl. The control values were obtained as in B. The depression of field e.p.s.p.s by 5 μl/ml and 10 μl/ml of Cerebrolysin was statistically significant ($p < 0.05$).

$GABA_A$ receptors were not involved. Activation of other inhibitory receptors (e.g., $GABA_B$), with glycine, taurine, and other amino acids being the possible agonist candidates, remains a possibility to be explored. Experiments with SAAS corresponding to the free amino acid composition of CB, suggest that its free amino acid fraction is probably not responsible for the delayed increase in synaptic responses, and that the peptide components may be responsible for the suppression and/or increase in field e.p.s.p.s. The calf blood hemodialysate was ineffective, suggesting that the brain-derived components present in CB solution possess distinct biological properties.

One can only speculate as to the relevance of this strong, inhibitory effect to the reported (8) therapeutic action of CB. A possible explanation is that reduced activity of excitatory synapses could prevent further injury of nerve cells by excitatory neurotransmitters. The delayed enhancement observed in some experiments and absent in experiments with SAAS was similar to that seen with applications of short chain peptides (4). Moreover, it has been shown recently that endogenous substances extracted from rabbit brain can have similar effects (10). The long-lasting enhancement of synaptic transmission in the hippocampus is of particular interest because of its similarity to the activity induced long-term potentiation (LTP) (11,13–15), which has been shown to be impaired in aged cortical structures (2,3). However, it remains unclear whether this small enhancement of synaptic transmission by CB is related to LTP.

In conclusion, these data suggest that the porcine brain hydrolysate CB containing low molecular weight peptides affects synaptic transmission in the rat hippocampus, which may be related to its reported behavioral effects and clinical efficacy.

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