Modulatory Role of Neuropeptides in Seizures Induced in Rats by Stimulation of Glutamate Receptors

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ABSTRACT Stimulation of glutamate receptors has been reported to modulate the expression of neuropeptides and their receptors in neurons. On the other hand, neuropeptides are known to regulate the presynaptic glutamate release and neuronal responses to excitatory neurotransmission. This evidence indicates a functional interaction between glutamatergic and neuropeptidergic transmission in the central nervous system (CNS). In this report, we provide pharmacologic evidence in experimental models of seizures, suggesting that somatostatin (SRIF) and neuropeptide Y (NPY) are endogenous modulators of glutamate-mediated hyperexcitability in the CNS. Electroencephalographic (EEG) and behavioral seizures were induced in rats by intrahippocampal or systemic injection of kainic acid, a glutamate analog. The number of EEG seizures and their total duration were inhibited significantly by intracerebral application of a SRIF receptor agonist. Similarly, kainic seizures were reduced by N\textsubscript{2}-(diphenylacetyl)-N\textsubscript{4}-(4-hydroxyphenyl)methyl-L-arginimide (BIBP 3226), a Y\textsubscript{1} receptor antagonist. Enhanced seizure susceptibility to pentylentetrazol, ensuing in rats after a systemic administration of kainic acid, was reduced significantly by intracerebral application of RC 160, a SRIF\textsubscript{1} receptor agonist, or NPY 13–36, a Y\textsubscript{2}/Y\textsubscript{5} receptor agonist. This evidence suggests that neuropeptide analogs may be of value for controlling seizures and possibly in other pathologic conditions associated with excessive glutamate function.

KEY WORDS: somatostatin receptors • neuropeptide Y receptors • epilepsy • neuropeptide Y • somatostatin • rats

Various neuropeptides have been identified in the central nervous system (CNS) where they coexist primarily with classical neurotransmitters (Hökfelt 1980). Functional evidence suggests that some neuropeptides act by inhibiting or facilitating classical neurotransmitter functions (Hökfelt 1980). In particular, recent evidence suggests that somatostatin (SRIF) and neuropeptide Y (NPY) affect synaptic transmission and neuronal excitability and that these effects are mediated significantly by their interaction with glutamatergic neurotransmission (Epelbaum 1986, Mancillas et al. 1986). Because an increase in glutamatergic function is involved in both seizure initiation and propagation in the CNS (Schwarcz and Meldrum 1985), it is of interest to investigate the functional role of SRIF and NPY in epilepsy and whether pharmacologic modifications of peptidergic function may control seizures by reducing glutamate action.

Recent electrophysiologic and biochemical findings have indeed shown that SRIF may act presynaptically by reducing glutamate release at hippocampal synapses (Boehm and Betz 1997), as well as postsynaptically by depressinig glutamate responses and baseline firing (Mancillas et al. 1986). These inhibitory effects appear to be mediated by the SRIF\textsubscript{1} family of receptors (Hoyer et al. 1995). Similarly, NPY reduces glutamate release acting on presynaptic Y\textsubscript{2} receptors (Klapstein and Colmers 1993), whereas an excitatory component of NPY appears to be mediated by postsynaptic Y\textsubscript{1} receptor subtypes (Brooks et al. 1987, Gariboldi et al. 1998).

Seizures in experimental models and humans profoundly affect the functional status of neuropeptide-containing neurons, particularly those neurons that also contain \(\gamma\)-aminobutyric acid (GABA) (DeLanerolle et al. 1989, Schwarzer et al. 1996, Sloviter, 1991) and induce the ectopic expression of NPY in glutamatergic granule cells of the dentate gyrus (Schwarzer et al. 1996, Sperk et al. 1992).

In this report, we describe the pharmacologic evidence showing that SRIF and NPY analogs, acting on specific receptor subtypes, inhibit seizures induced by glutamate receptor stimulation. This suggests that selective ligands for neuropeptide receptors may be of value for controlling excessive glutamate function.
MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats (225–250 g, Charles River, Calco, Italy) were used. Procedures involving animals and their care conformed with the institutional guidelines, in compliance with national and international laws and policies.

Electrode implantation, EEG recording and intracerebral injection of drugs. Surgical procedures for electrodes and injection cannula implantation have been described elsewhere (Vezzani et al. 1991). The procedures for recording the EEG and the intracerebral injection of drugs in unanesthetized rats have been described (Vezzani et al. 1991). The procedures for recording the EEG and the intracerebral injection of drugs in unanesthetized rats have been described (Vezzani et al. 1991).

Surgical procedures for electrodes and injection cannula implantation have been described elsewhere (Vezzani et al. 1991). Kainic acid (Sigma-Aldrich, St. Louis, MO; 0.04 mg in 0.5 μL PBS, pH 7.4) was infused (60 s) into the dorsal hippocampus in the region of granule cells. This was the smallest dose found to induce EEG seizures in all of the animals (Vezzani et al. 1991). Seizures consisted of high frequency and/or multispine complexes and/or high voltage synchronized spike or wave activity in cortical and hippocampal leads and provided a sensitive measure of the anticonvulsant activity of drugs (Vezzani et al. 1991). EEG recordings were made for at least 30 min to assess the spontaneous EEG pattern representing a control baseline period, then continuously for at least 180 min after kainic acid. Seizures were quantified by calculating the latency to the first seizure, the total number of seizures and the total time spent in seizures (the duration of all ictal episodes) during the EEG recording period.

Systemic injection of kainic acid and evaluation of seizures. Kainic acid or saline was injected subcutaneously into rats at a dose of 12 mg/kg. Rats showing repeated episodes of severe generalized limbic seizures with rearing and/or loss of postural control during the 3 h after drug injection were selected for further studies. These rats were tested for their increase in seizure susceptibility 30 d after kainic acid injection. A normally subconvulsive dose of pentylenetetrazol (30 mg/kg, intraperitoneal) was injected into rats and their behavior observed for 30 min. Myoclonic (all body twitch) convulsions were counted separately from generalized tonic-clonic seizures (tonic-clonic hindlimb extension with loss of posture). The onset time to the first seizure episode was also measured (Vezzani et al. 1991).

Schedule of treatment with peptide analogs. N-2-(diphenylacetamido)-N-[(4-hydroxyphenyl)methyl-d-arginamide] (BIBP 3226) (Rudolf et al. 1994; Dr. Karl Thomae GmbH, Biberach an der Riss, Germany) (5 and 10 μg in 1 μL) was dissolved in 25% polyethylene glycol and infused intrahippocampally at the same site as kainic acid injection. A normally subconvulsive dose of pentylenetetrazol (30 mg/kg, intraperitoneal) was injected into rats and their behavior observed for 30 min. Myoclonic (all body twitch) convulsions were counted separately from generalized tonic-clonic seizures (tonic-clonic hindlimb extension with loss of posture). The onset time to the first seizure episode was also measured (Vezzani et al. 1991).

RESULTS

Table 1 shows the anticonvulsant effects of BIBP 3226, a selective NPY Y1 receptor antagonist (Rudolf et al. 1994) and of SMS 201–995, an agonist at SRIF1 receptors (Reubi 1985), on EEG seizures induced by intrahippocampal kainic acid in rats. EEG seizures were measured in this study because they have been found to be the most significant, reproducible and quantifiable of the epilepsy-like sequelae after kainic acid administration in the rat hippocampus. EEG seizures provide a sensitive measure of the anticonvulsant activity of drugs (Vezzani et al. 1991).

BIBP 3226 (10 μg in 0.5 μL) significantly reduced the number of seizures and the time spent in seizures by two thirds (P < 0.01). BIBP 3226 (5 μg) decreased the time spent in seizures by 50% (P < 0.05), without significantly modifying the other seizure variables (data not shown). A similar, though less pronounced protective effect was achieved by injecting SMS 201–995 (5 μg in 0.5 μL) into the hippocampus. Thus, the number of seizures and their total duration were reduced by one third and one half, respectively (P < 0.05). Doses <5 μg were ineffective (not shown). SMS 201–995 reduced kainate-induced seizures more markedly when administered into the entorhinal cortex ipsilateral to the kainate injected hippocampus (though at a higher dose). Seizure parameters were reduced significantly by 60 and 75%, respectively (P < 0.01; see Table 1). The onset time to seizures was delayed significantly by BIBP 3226 (kainic acid, 12 ± 0.3 min; BIBP 3226, 33.4 ± 5.4 min, P < 0.01) and by 20 μg, but not 10 μg, SMS 201 995 in the entorhinal cortex (SMS 201–995, 39.8 ± 6.9 min, P < 0.01).

Table 2 shows the effects of NPY 13–36 and RC 160, which act as agonists at NPY Y1/Y5 and SRIF1 receptors (Reubi 1985) receptors (Michel et al. 1998), respectively, on the enhanced susceptibility to pentylenetetrazol seizures ensuing in rats 30 d after a systemic administration of kainic acid (Vezzani et al. 1994). Each compound reduced seizure susceptibility significantly by decreasing the number of rats showing tonic-clonic seizures (i.e., RC 160) or by increasing the number of rats without seizures (i.e., NPY 13–36). NPY 13–36 also significantly delayed the time to onset of seizures (P < 0.05).

DISCUSSION

Our results indicate that stimulation of SRIF1 and NPY Y1/Y5 receptors using preferential agonists reduces acute seizures and the chronically enhanced predisposition to generalized convulsions in rats. In addition, protection from acute seizures can be achieved by blocking NPY Y1 receptors selectively. Seizures in these models depend significantly on increased glutamatergic neurotransmission. Thus, our findings support previous electrophysiologic and biochemical evidence showing that SRIF and NPY interact functionally with glutamate in CNS.

SRIF has been reported to depress glutamate responses as well as baseline firing in both rat cortex and hippocampus (Mancillas et al. 1986) and to inhibit glutamate release acting...
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