BENZODIAZEPINE INVERSE AGONISTS AUGMENT LONG-TERM POTENTIATION IN CA1 AND CA3 OF GUINEA PIG HIPPOCAMPAL SLICES

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Summary—The effects of benzodiazepine inverse agonists on the long-term potentiation of synaptic transmission in hippocampal slices of the guinea pig were examined using an extracellular recording technique. Benzodiazepine inverse agonists, β-carboline-3-carboxylate (β-CCE), 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one (CGS-8216) and 2-[5-methylthien-3-yl]-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (S-135), augmented the magnitude of long-term potentiation induced by tetanic stimulation of input fibers in both the CA1 and the CA3 regions. β-Carbolene-3-carboxylate was more effective in augmenting long-term potentiation in CA1 than in CA3. Augmentation of long-term potentiation produced by β-CCE was antagonized by concomitant application of flumazenil, a benzodiazepine receptor antagonist. Therefore, the enhancing action of benzodiazepine inverse agonists on long-term potentiation, which is suggested to be a specific action, mediated by the GABA/benzodiazepine receptor complex, might help to explain the mechanism of the memory-enhancing effects of benzodiazepine inverse agonists, observed in some in vivo behavioral paradigms.

Key words—benzodiazepine inverse agonists, long-term potentiation, CA1 and CA3, hippocampal slice, guinea pig.

Benzodiazepines are known to enhance the function of the γ-aminobutyric acid (GABA) receptor/chloride channel complex by binding to specific recognition sites (benzodiazepine receptors) (Olsen, 1981; Polc, Bonetti, Schaffner and Haefely, 1982). γ-Aminobutyric acid is considered to be one of the important neurotransmitters regulating the memory and learning function (Izquierdo and Medina, 1991). Large doses of benzodiazepines have been reported to produce amnesia, like senile dementia, in humans (Lister, 1985) and in certain animal behavior paradigms (Sanger and Joly, 1985; Venault, Chapouthier, Carvalho, Simiand, Morre, Dodd and Rossier, 1986). On the other hand, benzodiazepine inverse agonists have affinities for benzodiazepine receptors, with pharmacological actions opposite to those of benzodiazepines (Jensen and Lambert, 1986; Kemp, Marshall, Wong and Woodruff, 1987). Benzodiazepine inverse agonists have been reported to attenuate amnesia produced by several drugs or surgical procedures (Kemp et al., 1987; Matsushita, Kawasaki, Matsubara, Eigyo, Shindo and Takada, 1988; Nagatani and Yamamoto, 1991). Furthermore, they can enhance the performance of memory and learning tasks (Duka, Stephens, Krause and Dorow, 1987; Emmanouil and Quock, 1990; Venault et al., 1986). However, the precise mechanisms behind the anti-amnesic and memory-enhancing effects of benzodiazepine inverse agonists remain unclear.

The long-term potentiation of synaptic transmission in the hippocampus is considered to be a possible synaptic model of the memory and learning process (Teyler and Discenna, 1984). Some benzodiazepines, which can produce amnesia in humans, have been reported to diminish long-term potentiation in slice preparations of the hippocampus (Riches and Brown, 1986; Satoh, Ishihara, Iwama and Takagi, 1986), which has a high density of GABA/benzodiazepine receptor complexes (Lloyd, Shemen and Hornykiewicz, 1977; Möhler and Okada, 1978; Pritchett, Sontheimer, Shivers, Ymer, Kettenmann, Schofield and Seeburg, 1989). Thus, the effect of benzodiazepine inverse agonists was examined on long-term potentiation in hippocampal slices. Satoh, Ishihara and Katsuki (1988) reported that antiracetam and piracetam, which have a similar effect on the memory and learning processes as benzodiazepine inverse agonists in behavioral paradigms (Merlini and Pinza, 1989) selectively augmented long-term potentiation at mossy fiber-CA3 synapses but not in the CA1 region (Satoh et al., 1986, 1988). In light of these findings, it should be interesting to compare the effects of benzodiazepine inverse agonists on long-term potentiation in CA1 and CA3 of the hippocampus. In this study, the
augmenting effects of benzodiazepine inverse agonists on long-term potentiation is demonstrated in CA1, as well as in CA3, of hippocampal slices of the guinea pig, which differs from the selective effects of nootropic drugs.

METHODS

Transverse hippocampal slices (450-500 μm thick) of the male guinea pig (200-350 g) were prepared, using a microslicer (DTK1000, Dosaka E.M.). Two slices were maintained at 34-35°C in a submersion recording chamber and perfused with artificial cerebrospinal fluid (artificial CSF; NaCl 124, KCl 5, KH₂PO₄ 1.24, MgSO₄ 1.3, CaCl₂ 2.4, NaHCO₃ 26, glucose 10 mM), saturated with 95% O₂ and 5% CO₂ at a flow rate of 5 ml/min.

Evoked field potentials were recorded extracellularly in the pyramidal cell layers of the CA1 or the CA3 region with a glass micropipette of 10 μm tip diameter, filled with 0.9% NaCl, following stimulation (rectangular pulse of 0.1 msec width at 0.2 Hz) of the Schaffer collateral/commissural pathway or mossy fibers, respectively, with a platinum bipolar electrode. The stimulus strength for producing half maximal response was used for each slice for single stimulation, as well as in the CA1 and CA3 regions (Fig. 3). Flumazenil alone, at a dose of 10⁻⁷ M, did not influence the height of the population spike (data not shown) and the magnitude of long-term potentiation in both the CA1 and CA3 regions (Fig. 3). Flumazenil alone, at a dose of 10⁻⁷ M, did not influence the height of the population spike (data not shown) and the magnitude of long-term potentiation in both the CA1 and CA3 regions (Fig. 3).

Effects of other benzodiazepine inverse agonists on long-term potentiation

Both S-135 and CGS-8216 are characterized pharmacologically as benzodiazepine inverse agonists by behavioral and electrophysiological experiments (Matsushita, et al., 1988). The drug S-135 significantly augmented the magnitude of long-term potentiation in the CA1 and the CA3 regions, at a dose of 10⁻⁷ M and 10⁻⁸ M, respectively (Fig. 4); S-135 was 10 times more effective in augmenting long-term potentiation in CA1 than in CA3 as observed with β-CCE. The dose–response relationships of S-135 on long-term potentiation in CA1 and CA3 showed characteristic reversed U shapes.

The drug CGS-8216 dose-dependently augmented long-term potentiation in CA3 at doses larger than 10⁻⁸ M (Fig. 5) (not tested in CA1).

Neither S-135 nor CGS-8126 influenced the evoked response in CA1 and CA3, prior to tetanic stimulation, as well as β-CCE.

DISCUSSION

The present study showed that benzodiazepine inverse agonists can augment the long-term potentiation of the Schaffer collateral/commissural fiber-CA1 synapse, as well as the mossy fiber-CA3 synapse, in contrast to nootropics which augment long-term potentiation only in CA3 but not that in CA1 (Satoh et al., 1986, 1988). Furthermore, the augmenting effects of benzodiazepine inverse agonists on long-
Effects of benzodiazepine inverse agonists on CA1 and CA3

Fig. 1. (A) Representative drawings of long-term potentiation observed in CA1 (left) and CA3 (right), produced by tetanic stimulation of input fibers. Upper rows show the long-term potentiation induced by tetanic stimulation without β-CCE (1 and 2); lower rows represent the long-term potentiation with β-CCE (3, 4 and 5). β-Carboline-3-carboxylate augmented long-term potentiation after tetanic stimulation but had little effect on the evoked field potentials prior to the tetanic stimulation in 4 (right and left). The magnitude of long-term potentiation at each time after tetanic stimulation was estimated as \( b/a \times 100 \) (%).

(B) Time-course of long-term potentiation after the tetanic stimulation, with or without β-CCE in the CA3 region. Abscissa shows time after the tetanic stimulation. Ordinate indicates the percentage of the amplitude of the population spike, compared with the mean of three averaged population spikes, prior to tetanic stimulation. Error bar shows standard error of mean. N = 6 (without β-CCE) and 4 (with β-CCE 10^{-8} M).

Fig. 2. Dose–response relationships of the augmenting effect of β-CCE on long-term potentiation (LTP) occurring in CA1 (○) and CA3 (□). Ordinate indicates the magnitude of long-term potentiation (mean ± SEM). **Significant increase (P < 0.01 vs without drug). N = 5–10 (CA1) and 3–6 (CA3) per group.

Fig. 3. Antagonism by flumazenil of the augmenting effect of β-CCE on long-term potentiation (LTP) occurred in CA1 and CA3. **Significant change (P < 0.01). N = 5–10 (CA1) and 3–6 (CA3) per group.
long-term potentiation were antagonized by concurrent application of flumazenil, indicating that this augmentation was mediated by an action on benzodiazepine receptors.

Induction of long-term potentiation in the Schaffer collateral/commissural fiber-CA1 synapse is mediated by the activation of N-methyl-D-aspartate (NMDA) receptors (Collingridge, Kehl and Mclennan, 1983) but not long-term potentiation in the mossy fiber-CA3 synapse (Harris and Cotman, 1986). The GABA/benzodiazepine receptor complex is present in large amounts and is widely distributed in the hippocampus (Lloyd et al., 1977; Möhler and Okada, 1978; Pritchett et al., 1989) and GABA is known to play a role in hyperpolarizing pyramidal cells as an inhibitory transmitter in both the CA1 and CA3 regions (Andersen, Bie and Ganes, 1982; Janigro and Schwartzkroin, 1988). Hyperpolarization of pyramidal neurons leads to diminished induction of long-term potentiation in CA1 (Malinow and Miller, 1986), possibly by reducing activation of NMDA receptors (Collingridge, Kehl and McLennan, 1983) but not long-term potentiation in the hippocampus (Lloyd et al., 1977; Möhler and Okada, 1978; Pritchett et al., 1989) and GABA is known to play a role in hyperpolarizing pyramidal cells as an inhibitory transmitter in both the CA1 and CA3 regions (Andersen, Bie and Ganes, 1982; Janigro and Schwartzkroin, 1988). Hyperpolarization of pyramidal neurons leads to diminished induction of long-term potentiation in CA1 (Malinow and Miller, 1986), possibly by reducing activation of NMDA channels (Mayer, Westbrook and Guthrie, 1984) and in CA3 perhaps by decreasing the Ca²⁺ influx through a Voltage-dependent Ca²⁺ channel (Williams and Johnston, 1989). In fact, benzodiazepines depress long-term potentiation in the hippocampus by potentiating GABA-mediated inhibition (Riches and Brown, 1986; Satoh et al., 1986). Based on these findings, benzodiazepine inverse agonists could augment long-term potentiation in both the CA1 and the CA3 regions by decreasing GABAergic inhibition.

These mechanisms of action differ from that of nootropics, which potentiate the cholinergic function in the CA3 region (Spignoli and Pepeu, 1986; Satoh et al., 1988).

β-carboline-3-carboxylate and S-135 were 10–100 times more effective in augmenting long-term potentiation in CA1 than in CA3. The easier occurrence of long-term potentiation and/or greater participation of a GABAergic influence on the induction of long-term potentiation in CA1, than in CA3 might explain the different efficacies of benzodiazepine inverse agonists on long-term potentiation in the CA1 and the CA3 regions. In some behavioral paradigms, β-CCM, which is a pharmacologically similar compound to β-CCE, has memory-enhancing effects (Venault et al., 1986). It has been reported that S-135 attenuates amnesia, produced by several procedures in behavioral paradigms, using rats and mice (Matsushita et al., 1988). Furthermore, other benzodiazepine inverse agonists have been shown to cause a nootropic-like action in human volunteers (Duka et al., 1987) and in animals (Emmanouil and Quock, 1990). Moreover, the dose–response relationships of S-135 on long-term potentiation in the present study were likely to have the reversed U shape, as observed in some in vivo memory and learning task paradigms (Matsushita et al., 1988; Venault et al., 1986), suggesting that moderate activation of neurons by a benzodiazepine inverse agonist, with moderate intrinsic activity, is necessary and adequate for enhancing the memory and learning process. In fact, S-135 has greater intrinsic activity than β-CCE and CGS-8216 (Matsushita et al., 1988). In contrast, benzodiazepines diminish long-term potentiation in the hippocampus (Riches and Brown, 1986; Satoh et al., 1986) and also produce amnesia in humans (Lister, 1985) and in animals (Sanger and Joly, 1985; Venault et al., 1986). Based on these findings, it is suggested that the memory-enhancing and anti-amnesic effects of benzodiazepine inverse agonists, observed in in vivo behavioral paradigms, are partly mediated by a direct action on the hippocampus. Benzodiazepine inverse agonists might be memory enhancers or cognitive improvers, with a pharmacologically different mechanism from that of nootropics.

REFERENCES


Effects of benzodiazepine inverse agonists on CA1 and CA3


