Benzodiazepines: GABA and glycine receptors on single neurons in the rat medulla

A. DRAY*, D. W. STRAUGHAN, *Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, U.K.*

On the basis of indirect *in vivo* experiments and observations using isolated cns receptor preparations the actions of benzodiazepines have been linked with two putative inhibitory amino acid neurotransmitters, GABA (Polc, Mohler & Haefely, 1974; Costa, Guidotti & others, 1975; Suria & Costa, 1975; Haefely, Kulcsar & others, 1976) and glycine (Young, Zukin & Snyder, 1974; Snyder, 1975).

However, little is known concerning the direct effects of benzodiazepines on the activity of single central neurons and no direct evaluation has been made of their actions compared with those of GABA or glycine in the central nervous system. In this report the effects of two benzodiazepines, chlordiazepoxide and flurazepam, have been compared with the effects of GABA and glycine administered directly to the same central neurons by microiontophoresis. In addition two antagonists, bicuculline methochloride and strychnine (Hill, Simmonds & Straughan, 1973; Dray, 1975) have been used to determine whether flurazepam interacts with GABA or glycine receptors.

Experiments were performed on adult albino rats anaesthetized with urethane (1·4 g kg^{-1}, i.p.) and prepared for brain-stem recording by the method of Bradley & Dray (1973). Extracellular action potentials were recorded from single spontaneously active neurons in the medulla, using one barrel of a multibarrel glass micropipette. Other barrels were filled with substances for electrophoresis into the immediate vicinity of neurons. Recordings were made using conventional techniques. Drugs for electrophoresis were made up as follows: GABA, 0·2M, pH 3·5; glycine 0·2M, pH 3·5; acetylcholine 0·2M, pH 4·0; sodium glutamate, 0·2M, pH 8·5: chlordiazepoxide 0·2M, pH 4·0; flurazepam 0·2M, pH 4·0; bicuculline methochloride 5mM, pH 3·5; strychnine 5mM, pH 3·5.

Direct effects of benzodiazepines. Chlordiazepoxide (10–200nA) or flurazepam (3–200nA) was tested on cells whose activity could be depressed by GABA and glycine. Both benzodiazepines generally reduced cell firing in a dose dependent manner. Flurazepam reduced the activity of all cells tested (30) whereas the effects of chlordiazepoxide were less constant, 6 of 11 cells being depressed and 2 excited. Two types of depressant response could be observed, one with a relatively short onset and rapid recovery (21/30 with flurazepam: 5/6 with chlordiazepoxide) resembling the effects of GABA or glycine, and less commonly, a depression with longer onset and which outlasted the period of ejection by some 2–3 min. The effects produced by each compound could be clearly distinguished from the effects of Na^+ ejection to control for current artifacts. The effects were reproducible with no evidence of tachyphylaxis or spike size depression. Both flurazepam and chlordiazepoxide were approximately half as potent as GABA or glycine when the currents required to produce the same magnitude of depression, within the same period of ejection, were compared.

Interactions with putative transmitters. Reproducible responses to GABA and glycine, or acetylcholine and glutamate were obtained during consecutive applications of these compounds during a fixed time sequence. Their effects were tested during the continued administration of chlordiazepoxide or flurazepam from another barrel of the same micropipette at a rate (3–20nA) which produced minimal effects on spontaneous background firing. Although a gradual depression of background firing was eventually produced, no selective interactions could be shown with depression produced GABA or glycine, or excitation by acetylcholine or glutamate. Thus chlordiazepoxide reduced the effects of both GABA and glycine in only 1 of 7 cells tested and flurazepam did not modify GABA or glycine depressions (5 cells). On the other hand chlordiazepoxide (2/3 cells) and flurazepam (3/3 cells) reduced acetylcholine and glutamate excitations and this appeared to parallel the changes produced by the benzodiazepines in the spontaneous background firing.

Interactions with bicuculline methochloride or strychnine. On neurons where comparable, reproducible responses to glycine, GABA and flurazepam were obtained bicuculline methochloride (5–25nA) consistently antagonized GABA (6/8 cells) and flurazepam depressions (5/8 cells) without affecting glycine depression (0/8 cells) (Fig. 1A). In contrast strychnine (2–10nA) selectively reduced or abolished inhibition by glycine in all cells tested (7 cells) at a time when the effects of GABA (0/7 cells) or flurazepam (1/7 cells) were not modified (Fig. 1B). Strychnine abolished GABA and flurazepam responses (3 cells) only at doses far in excess of those required to selectively antagonize glycine.

Previous experiments using microelectrophoresis of a sparingly soluble benzodiazepine, nitrazepam, failed to show depression of cell firing though changes in the firing patterns of hippocampal and lateral geniculate neurons were noted (Steiner & Hummel, 1968). However in the present experiments two water soluble

*Correspondence

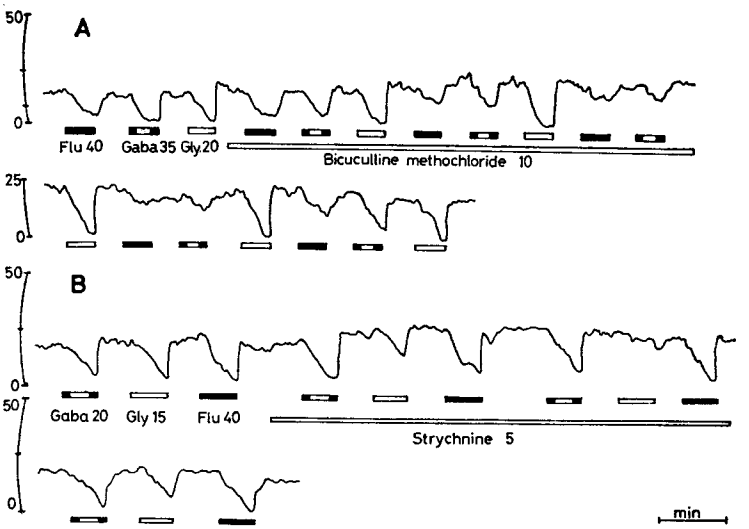

FIG. 1. Continuous rate-meter records (spikes s^{-1} against min) of a spontaneously active neuron in the medulla showing the reversible effects of bicuculline methochloride and strychnine. Expelling currents (nA) are indicated next to the applications. A. Bicuculline methochloride reduced the depressant responses to GABA and flurazepam (Flu) but not the responses to glycine. B. Strychnine selectively abolished the effects of glycine but not depressions by GABA or flurazepam.

benzodiazepines, chlordiazepoxide and flurazepam, typically depressed neuronal activity, though chlordiazepoxide occasionally excited cells. No selective interactions of chlordiazepoxide or flurazepam were observed with the responses of neurons to GABA, glycine, acetylcholine or glutamate. Both compounds, however, reduced neuronal sensitivity to acetylcholine and glutamate.

Further studies with flurazepam, the compound which in the present experiments produced most consistent effects, showed that its depressant effects were never selectively modified by strychnine but consistently reduced by bicuculline. This provides direct support for the involvement of benzodiazepines with GABA rather than glycine mediated processes. However it remains to be demonstrated whether this action of benzodiazepines on GABA receptors is relevant to their therapeutic effectiveness.

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