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In-vitro release of [^{14}C]glutamate from dentate gyrus is modulated by GABA

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The effect of GABA (10^{-3} and 10^{-4} M) on the release of preloaded [^{14}C]glutamate from slices of rat dentate gyrus, in response to K^+ stimulation, was studied in Ca^{2+}-free and normal Krebs solutions. Release in Ca^{2+}-free solution was significantly enhanced, but there was no change in release in normal Krebs solution. These results imply that Ca^{2+}-dependent (presumably neuronal) release of glutamate from the dentate gyrus is depressed by GABA, while non-neuronal Ca^{2+}-independent release is enhanced.

There is a great deal of evidence available concerning the post-synaptic actions of the inhibitory amino acid, γ-aminobutyric acid (GABA) in the hippocampus (Krnjevic 1976; Alger & Nicoll 1982; Andersen et al 1982). The presynaptic actions of GABA are less well documented, although it has been shown to affect transmitter release in the hippocampus (Baba et al 1983; Fung & Fillenz 1983), as in several other areas of the brain (Bowery et al 1980; Schlicker et al 1984). Our interest in GABA-mediated modulation of transmitter release derives from two recent observations suggesting that long-term potentiation (LTP) in the hippocampus may be under inhibitory control: GABA antagonists reduce the threshold for LTP (Wigström & Gustafsson 1983a, b), and tetanic stimulation of the inhibitory commissural input to the dentate gyrus blocks the induction of LTP in the perforant path (Douglas et al 1982). These results have generally been interpreted in terms of a postsynaptic locus of control over LTP. However, we have recently found that commissural stimulation also blocks the increase in glutamate release which is associated with LTP in the perforant path (Bliss et al 1985), an observation which raises the possibility that the commissural pathway exerts its blocking effect by a presynaptic action. As a first step in the pharmacological analysis of this phenomenon, we have examined the effect of GABA on K^+-induced release of preloaded [^{14}C]glutamate ([^{14}C]glu) from slices of dentate gyrus.

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Methods

Male Sprague Dawley rats (250–300 g) were killed by stunning and decapitation. The brain was rapidly removed and the dentate gyrus separated from the hippocampus proper. Slices (0·2 mm thick) were prepared using a McIlwain tissue chopper, suspended in 2 ml oxygenated Krebs solution containing 20 μl [^{14}C]glu (280 μCi mmol^{-1}, Amersham, UK; final concentration 2×10^{-5} M), and incubated at 37 °C for 20 min. The tissue suspension was divided among 8 tubes, centrifuged at 5000 rev min^{-1} for 4 min at 4 °C and the supernatant discarded. These conditions were employed in all subsequent centrifugation steps. The pellet was resuspended in 2 ml ice-cold normal or Ca^{2+}-free Krebs solution containing 1 mM EGTA, and centrifuged. This rinsing procedure was repeated 4 times, at which stage basal release of radiolabel had reached a steady baseline, as shown in preliminary experiments. The pellet was then resuspended in 2 ml normal or Ca^{2+}-free Krebs solution, incubated at 37 °C for 5 min and centrifuged. Aliquots (0·25 ml) of supernatant were retained for scintillation counting to give an estimate of basal release of [^{14}C]glu. Release was stimulated by repeating the incubation procedure in 2 ml normal or Ca^{2+}-free Krebs solution to which 40 mM K^+ had been added. After the subsequent centrifugation step, 0·25 ml of supernatant was retained for estimating K^+-induced release. Finally the slices were incubated in 2 ml normal or Ca^{2+}-free Krebs solution, centrifuged, and 0·25 ml of supernatant retained to check that [^{14}C]glu release had returned towards baseline. Each aliquot and the drained tissue slices were added to 5 ml scintillant (Beckman, EP) and counted for 5 min. In some experiments GABA (10^{-3} or 10^{-4} M) was added, either alone or in the presence of bicuculline (10^{-4} M), muscimol (10^{-4} M) or baclofen (10^{-4} M), to Krebs solution containing 40 mM K^+. Release was studied in the presence and absence of Ca^{2+}, and the effect of the drugs alone was also examined. Aliquots of supernatant were taken from about half of the experi-

ments (randomly chosen) for separation of radiolabel into [^{14}C]glu and [^{14}C]GABA by ion-exchange chromatography (Dolphin 1982); results of these experiments indicated that 70–80% of released ^{14}C was recovered as [^{14}C]glu and the remainder as [^{14}C]GABA. Recovery of [^{14}C]glu was similar in all experiments demonstrating that the various experimental conditions did not significantly alter metabolism of glutamate. Total radioactivity accumulated by the tissue slices was estimated as the sum of basal, stimulated and post-stimulation release together with residual radioactivity in the slices. Results are expressed as a percentage of the total radioactivity in the tissue at the beginning of the incubation period.

Results

Tissue accumulation of [^{14}C]glu was similar in all groups. The presence of Ca^{2+} in the incubation medium did not significantly alter basal release: mean basal release was 1·93% (±0·14 s.e.m.) in the presence of Ca^{2+}, and 1·92% (±0·12) in the absence of Ca^{2+}. Mean fractional release of [^{14}C]glu from slices was significantly increased when 40 mM K^+ was added to the incubation medium: in Ca^{2+}-free medium, release was 2·53% (±0·17), while in the presence of Ca^{2+} it increased to 5·39% (±0·39). Since basal release was similar in all treatment groups, results hereafter will be presented as net K^+-induced release (i.e., stimulated release minus basal release).

GABA significantly enhanced Ca^{2+}-independent release of [^{14}C]glu ($P < 0·01$). In the presence of Ca^{2+}, however, release was unchanged (Fig. 1). Consequently, there was a net decrease in the Ca^{2+}-dependent component of release (calculated as the difference between release in the presence and absence of Ca^{2+}). The $GABA_A$ agonist muscimol (10^{-4} M) led to a similar decrease in Ca^{2+}-dependent release ($P < 0·05$), but the presence of GABA did not further enhance the action of muscimol (Fig. 2). The $GABA_B$ agonist, baclofen, also mimicked the effect of GABA. Ca^{2+}-dependent release of [^{14}C]glu was significantly decreased compared with controls ($P < 0·01$) but in this case the effect of baclofen was further potentiated by the presence of GABA, 10^{-3} M (Fig. 2). The $GABA_A$ antagonist, bicuculline, was examined for its effect on release, and for its ability to antagonize GABA-mediated changes in release. Bicuculline (10^{-4} M) decreased Ca^{2+}-dependent release of [^{14}C]glu but failed to antagonize the effect of GABA (10^{-3} or 10^{-4} M; Fig. 2).

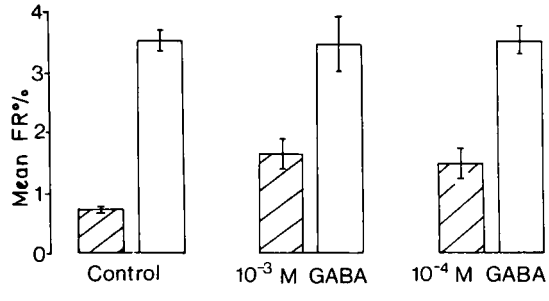

FIG. 1. Effect of GABA (10^{-3} and 10^{-4} M) on K^+-induced release of [^{14}C]glu from dentate slices. Results given are values for mean fractional release (FR) of [^{14}C]glu expressed as a percentage. Release of [^{14}C]glu was stimulated by addition of 40 mM K^+ to normal (plain columns) or Ca^{2+}-free (hatched columns) Krebs solution. Release in the presence of Ca^{2+} was significantly greater than in its absence ($P < 0·01$). GABA (10^{-3} or 10^{-4} M), when added to the incubation medium, significantly increased release in the absence of Ca^{2+} ($P < 0·01$) but had no effect on release in the presence of Ca^{2+}.

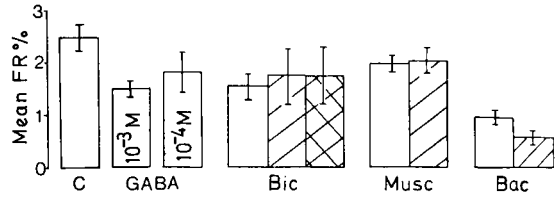

FIG. 2. Effect of GABA and GABA-ergic agents on K^+-stimulated Ca^{2+}-dependent [^{14}C]glu release from dentate slices. GABA [10^{-3} (hatched columns) or 10^{-4} M (cross-hatched columns)] significantly reduced mean fractional release (FR) of [^{14}C]glu compared with control (C; $P < 0·05$). This effect was mimicked by muscimol (Musc; 10^{-4} M) and baclofen (Bac; $P < 0·05$, compared with control) and the effect of baclofen was potentiated in the presence of GABA (10^{-3} M). Bicuculline (Bic; 10^{-4} M) also decreased release but failed to antagonize the effect of GABA.

Discussion

We have calculated neuronal release as the difference between the release of [^{14}C]glu in the presence and absence of Ca^{2+}, on the assumption that depolarization-evoked neuronal release is Ca^{2+}-dependent and that release from non-neuronal elements is Ca^{2+}-independent. There is considerable evidence suggesting that this is the case (Balcar et al 1977; Fonnum 1984; Sellström & Hamberger 1977). Given this assumption, our results indicate that neuronal release of [^{14}C]glu from dentate slices is under the inhibitory control of GABA. Further evidence for a presynaptic action of GABA in the hippocampus has recently been provided by experiments showing that K^+-induced release of [^{3}H]glu from slices of whole hippocampus is depressed by GABA (Baba et al 1983). Similarly, GABA has been found to depress the release of [^{3}H]noradrenaline from hippocampal synaptosomes (Fung & Fillenz 1983). Release in the presence and absence of Ca^{2+} was not examined in the latter studies, so that direct comparisons with our results cannot be made. Indeed, our conclusion that GABA decreases Ca^{2+}-dependent release derives from an *increased* release in the absence of Ca^{2+}, coupled with a *lack* of effect in the presence of Ca^{2+}.

The effect of GABA was mimicked both by the $GABA_A$ agonist, muscimol and, to a somewhat greater extent, by the $GABA_B$ agonist, baclofen. While the effect of muscimol was not affected by the presence of

GABA, the effect of baclofen was potentiated. Bicuculline, the $GABA_A$ antagonist, was ineffective in reversing the decrease in [^{14}C]glu release produced by GABA, but alone it significantly reduced Ca^{2+}-dependent release. These latter findings contrast with the reported absence of presynaptic activity of GABA antagonists in whole hippocampal slices (Baba et al 1983), or in cortical or striatal slices (Schlicker et al 1984). The apparent discrepancy is probably due to the fact that no allowance was made by previous workers for changes in Ca^{2+}-independent release and it was a striking feature of our experiments that GABA, muscimol, baclofen and bicuculline all potentiated the release of [^{14}C]glu in Ca^{2+}-free conditions. These findings suggest that release from non-neuronal elements, presumably glia, may also be modulated by GABA. We should also emphasize that this effect is unlikely to be non-specific, since a similar potentiation was never seen in a series of parallel studies on the modulatory effects of noradrenaline and noradrenergic agents (Lynch & Bliss 1986).

In this study, the concentration of GABA used was either 10^{-3} or 10^{-4} M. It could therefore be argued that interaction with specific receptors may not have occurred because of the relatively high concentration. However, very high concentrations of GABA (up to 10 mmol kg^{-1}) have been measured in various brain areas (Okada et al 1971) which probably explains the high concentrations typically used in electrophysiological experiments (Konnerth & Heinemann 1983).

It should be considered whether the changes in glutamate uptake which may occur under the various experimental conditions of the present study could contribute to our findings. However, it has been shown that accumulation of [^{3}H]glutamate into either cerebral cortex or spinal cord preparations is unaffected by incubation with concentrations of GABA as high as 10^{-3} M (Logan & Snyder 1972).

Pharmacological evidence, in particular the finding that baclofen, a specific $GABA_B$ agonist, mimics the effect of GABA in decreasing transmitter release (Bowery et al 1980), indicates that the presynaptic effects of GABA are mediated by $GABA_B$ receptors (Fung & Fillenz 1983). In the present experiments, baclofen was also found to mimic the effect of GABA and this finding, together with the finding that bicuculline failed to antagonize the effect of GABA, suggests that presynaptic inhibition in the dentate gyrus is also likely to be mediated by $GABA_B$ receptors. The mechanism of presynaptic inhibition may involve a blockade of Ca^{2+} entry by GABA (Dunlap & Fischbach 1981; Konnerth & Heinneman 1983), and our finding that GABA decreased Ca^{2+}-dependent release is consistent with this suggestion.

In the dentate gyrus, and in hippocampal area CA1, application of either bicuculline or picrotoxin facilitates the induction of LTP (Wigström & Gustafsson 1983a, b). Moreover, tetanic stimulation of the commissural input to the dentate gyrus, immediately before the tetanus to the perforant path, blocks the induction of LTP, probably by a disinhibitory mechanism involving GABAergic interneurons (Douglas et al 1983). The most straightforward interpretation of these results is that the threshold for LTP is under postsynaptic inhibitory control. In providing evidence for the existence of presynaptic GABA receptors in the dentate gyrus, the present results raise the alternative possibility that GABA-related modulation of LTP may be presynaptically rather than postsynaptically mediated.

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