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EFFECTS OF ω-AMINO ACIDS ON TRITIATED DOPAMINE RELEASE FROM RAT STRIATUM: EVIDENCE FOR A POSSIBLE GLYCINERGIC MECHANISM

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Abstract—The effects of GABA and glycine on the release of tritiated dopamine from prelabelled slices of rat striatum have been compared. Both GABA (>50 μM) and glycine (>200 μM) released tritiated dopamine, but had no effect on the release of radiolabelled 5-hydroxytryptamine and GABA. The GABA antagonist picrotoxin (50 μM) markedly reduced the ability of GABA to release [^{3}H]dopamine, but had no effect on the glycine response. Conversely strychnine (0.5 μM), a specific glycine receptor antagonist at low concentrations, abolished both the GABA and the glycine response on [^{3}H]dopamine release. Two other ω-amino acids, β-alanine and taurine, both at 500 μM, had no effect on [^{3}H]dopamine release from rat striatal slices.

In additional experiments, release of radioactivity was demonstrated from neonatal rat spinal cord and striatal slices after prelabelling with [^{3}H]glycine. This release was calcium-dependent. The possibility that glycine may function as a neurotransmitter substance within the striatum is considered, and the hypothesis that GABA may partially exert some of its pharmacological effects through the glycine receptor is discussed.

The neostriatum of the rat contains high concentrations of the putative neurotransmitter dopamine (DA). The origin of the striatal DA nerve terminals is the zona compacta of the midbrain substantia nigra [1]. However, the neostriatum is also rich in a number of other putative neurotransmitter candidates [2] and there have been many studies to investigate how these transmitters are able to influence striatal DA release. For instance glutamic acid [3], γ-amino-n-butyric acid (GABA) [4, 5] and substance P [6], can stimulate release of preloaded [^{3}H]DA release from striatal slices, whereas opiates and enkephalins can inhibit striatal dopamine release [7]. A puzzling anomaly in these studies has been reported by Starr [8], who noted that while GABA can stimulate [^{3}H]DA release from striatal slices, the putative GABA agonists, 3-aminopropane sulphonic acid, γ-hydroxybutyrate and baclofen were ineffective. We have recently provided evidence along with others [9, 10] that glycine may be a neurotransitter in the substantia nigra. Furthermore, we have shown that glycine is able to stimulate the release of [^{3}H]DA from dendrites of rat substantia nigra [9]. It is known that GABA can exert agonist properties at spinal glycine receptors [11]. We therefore decided to compare the effects of GABA and glycine on the release of [^{3}H]DA from rat striatal slices, first to try to determine whether glycine can also stimulate [^{3}H]DA release from striatum, and second, to discover whether some of the effects of GABA may be explained in terms of glycine receptor activation.

To test the specificity of these effects the action of GABA and glycine was also studied on the release of preloaded [^{3}H]-5-hydroxytryptamine (5-HT) and [^{3}H]GABA from rat striatal slices. As a further test of specificity the effect of the other ω amino acids, taurine and β-alanine, was studied on efflux of [^{3}H]DA. In addition we have compared the release of [^{3}H]glycine from slices of adult and neonatal striatum to that released from adult and neonatal spinal cord in an attempt to provide evidence that glycine may be released from nerve terminals in the striatum.

MATERIALS AND METHODS

Female Porton rats weighing approx. 200 g were used in this study. Rats were killed by cervical dislocation, the brains removed and further dissection carried out over ice. The neostriatum was dissected out from coronal sections of the forebrain. Central portions of left and right striata were pooled with those from three other animals and the tissue was chopped in two directions at 0.2 mm intervals on a McIlwain tissue chopper.

The methods used to study the release of radioactive transmitter from prelabelled brain slices have been described in detail elsewhere [12]. The striatal slices were preincubated for 20 min in 2 ml oxygen-bubbled Krebs–Ringer bicarbonate buffer (pH 7.4) containing labelled transmitter at a final concentration of 10^{-8} M ([^{3}H]GABA, sp. act. 54 Ci/mM; [^{3}H]DA, 5.0 Ci/mM; [^{3}H]-5-HT, 13 Ci/mM; [^{3}H]glycine, 23 Ci/mM; Radiochemical Centre, Amersham) at 37°. Aminooxyacetic acid (Sigma; 10 μM) or pargyline (Sigma; 50 μM) were present to inhibit metabolism of GABA or DA and 5-HT respectively; ascorbic acid (0.1%; Sigma) was present to inhibit oxidation of DA and 5-HT. The prelabelled tissue was transferred to superfusion chambers and a half-hour washout period was commenced by superfusing with Krebs at a rate of 0.5 ml/min. After 30 min, 1 ml fractions were collected every 2 min and radioactivity was determined before and after addition of 50 mM KCl for 4 min or test drug, either alone or in conjunction with 20 mM KCl, perfused for 8 mins. Amino acids investigated on release of radioactivity were glycine (200–400 μM; Fisons), GABA (50–400 μM; Sigma), taurine (500 μM; Sigma) and β-alanine (500 μM; Sigma). When antagonists were included

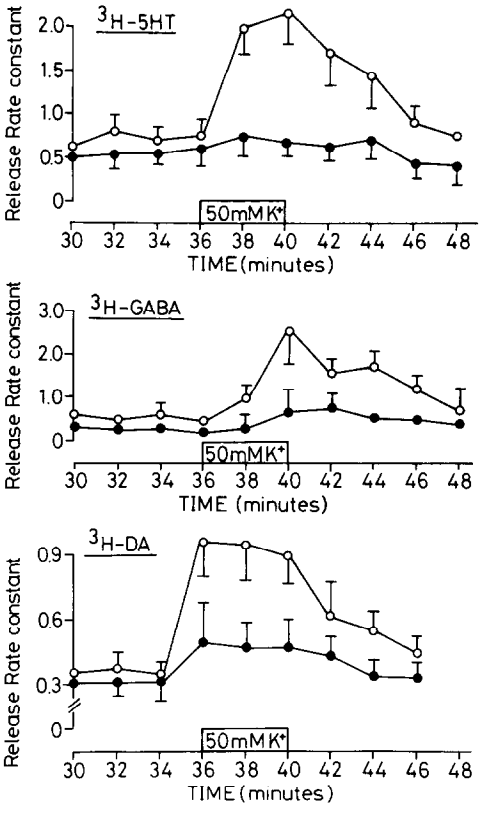

Fig. 1. Calcium-dependent potassium-evoked (50 mM KCl) release of radioactivity from superfused slices of adult rat striatum prelabelled *in vitro* with 10^{-8} M [^{3}H]-5-HT, [^{3}H]GABA or [^{3}H]dopamine ([^{3}H]DA). Open circles represent control K^+ stimulation as indicated by the bar: 50 mM K^+ was added for 4 min, 36 min after the start of superfusion. Closed circles show experiments performed in the absence of calcium (1 mM Mg^{2+}, <0.1 mM Ca^{2+}). The results are shown as a rate constant derived from the recovered radioactivity and expressed as a percentage of total radioactivity in the tissue at that instant. Each point is the mean of four or eight determinations. Vertical bars denote S.E.M. Conventional chromatographic techniques showed that the radioactivity in the superfusate was predominantly unchanged transmitter.

in the experiment these were present throughout the superfusion period. Antagonists used were strychnine hydrochloride (0.5 μM; Sigma) and picrotoxin (50 μM; Sigma). The effect of replacing the calcium concentration in the superfusing medium with magnesium chloride (1 mM) was studied on the K^+-evoked release of labelled transmitter.

In additional experiments the releasing effect of 50 mM KCl was studied in slices of striatum and spinal cord (thoracic and lumbar regions) taken from 2-day-old and adult rats and preloaded with [^{3}H]glycine. The effect of glycine (200 μM) on the release of [^{3}H]DA from two-day-old striatal slices was also studied.

RESULTS

Calcium-dependent, potassium-evoked release of labelled transmitter from striatal slices. A depolarizing stimulus (50 mM KCl) increased the rate of efflux of [^{3}H]-5-HT, [^{3}H]DA and [^{3}H]GABA from prelabelled slices of rat striatum. In all cases this effect of KCl was markedly reduced in a low-calcium, high magnesium medium (Fig. 1). 50 mM KCl also stimulated the rate of efflux of preloaded [^{3}H]glycine from 2-day-old rat striatal and spinal cord slices but not from adult striatal or spinal cord slices. This effect of K^+ on neonatal striatal and spinal cord slices was reduced in low-calcium, magnesium-substituted medium (Fig. 2).

Specific stimulation of [^{3}H]DA efflux by GABA and glycine from striatal slices. Glycine at doses above 200 μM and GABA above 50 μM both caused a marked stimulation in the basal efflux of [^{3}H]DA (Fig. 3). At 1 mM neither GABA nor glycine had any effect on the efflux of [^{3}H]-5-HT or [^{3}H]GABA (Fig. 3). At 200 μM GABA and glycine were equally effective at potentiating [^{3}H]DA release evoked by 20 mM KCl (Fig. 4). Picrotoxin (50 μM) caused a marked and significant reduction in the ability of GABA to stimulate [^{3}H]DA release but had no effect on glycine's ability to stimulate [^{3}H]DA efflux (Fig. 5a). On the other hand, strychnine hydrochloride (0.5 μM) caused a total and significant abolition of both the GABA (400 μM) and glycine (400 μM) evoked release of [^{3}H]DA (Fig. 5b).

Neither strychnine nor picrotoxin alone at the stated doses had any effect on the unstimulated basal efflux of [^{3}H]DA. Of the other amino acids studied, both taurine and β-alanine were ineffective at evoking [^{3}H]DA release at concentrations of 500 μM.

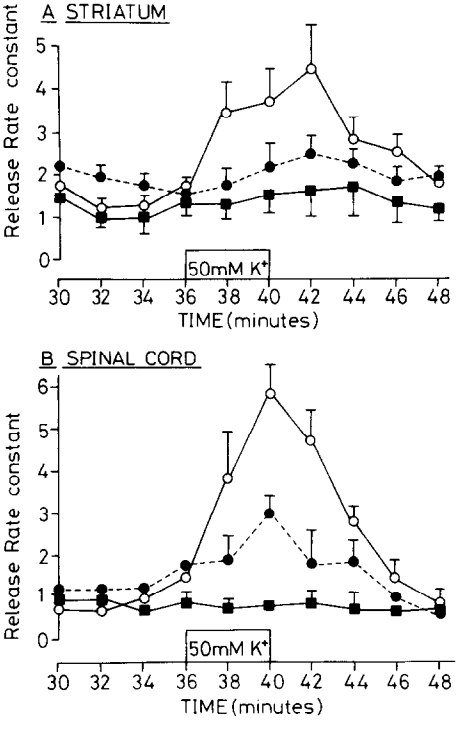

Fig. 2. Potassium-evoked (50 mM KCl) release of radioactivity from superfused slices of adult and neonatal rat striatum (A) and spinal cord (B) prelabelled with 10^{-8} M [^{3}H]glycine *in vitro*: see legend to Fig. 1 for experimental details. ○—○, Release of radioactivity from neonatal slices; ●- - -●, release of radioactivity from neonatal slices in the absence of calcium (1 mM Mg^{2+}), ■—■, release of radioactivity from adult rat slices.

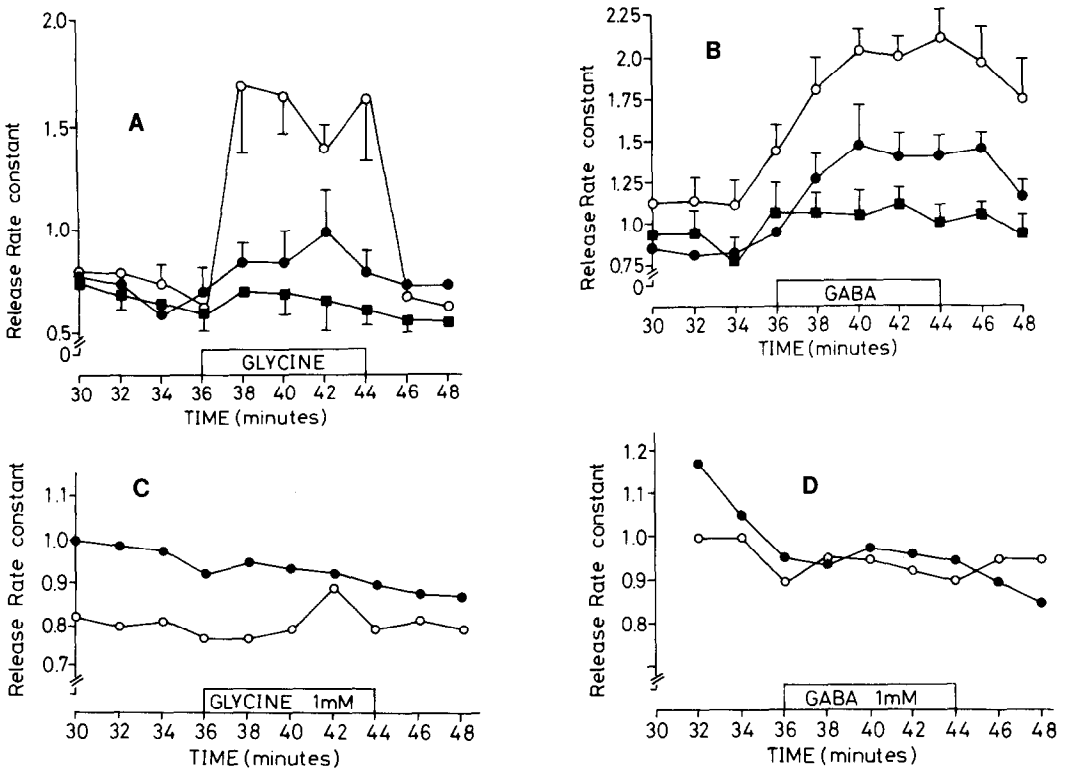

Fig. 3. A. Effect of glycine on the efflux of radioactivity from superfused slices of adult rat striatum, prelabelled *in vitro* with 10^{-8} M [^{3}H]dopamine. As indicated by the bar, glycine was added for 8 min, 36 min after the start of superfusion. Concentrations of glycine used were 200 μM (■—■), 300 μM (●—●) and 400 μM (○—○). Results are shown as described in the legend to Fig. 1. B. Effect of GABA on the efflux of radioactivity from superfused slices of adult rat striatum prelabelled *in vitro* with 10^{-8} M [^{3}H]dopamine. Concentrations of GABA used were 50 μM (■—■), 200 μM (●—●) and 400 μM (○—○). For further details see legend to Fig. 3A. C. Effect of 1 mM glycine on the efflux of radioactivity from superfused slices of adult rat striatum prelabelled *in vitro* with [^{3}H]GABA (●—●) or [^{3}H]-5-HT (○—○). Standard errors are omitted for clarity. For further details see legend to Fig. 3A. D. Effect of 1 mM GABA on the efflux of radioactivity from superfused slices of rat striatum prelabelled *in vitro* with [^{3}H]GABA (●—●) or [^{3}H]-5-HT (○—○). Standard errors are omitted for clarity. For further details see legend to Fig. 3A.

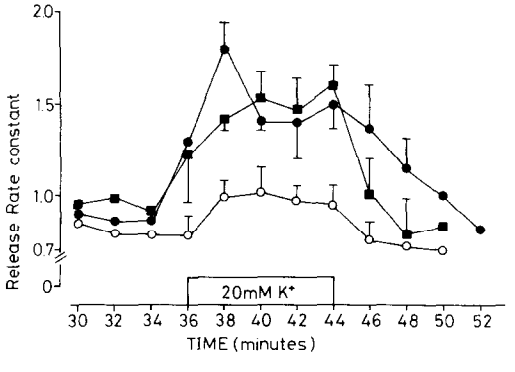

Fig. 4. Effect of 200 μM GABA (●—●) and 200 μM glycine (■—■) on the K^+-evoked efflux of radioactivity from superfused slices of adult rat striatum prelabelled with [^{3}H]dopamine *in vitro*. As indicated by the bar, potassium (20 mM KCl) and test drug were added for 8 min, 36 min after the start of superfusion. Open circles show control stimulation by 20 mM K^+ alone. Results are shown as described in the legend to Fig. 1.

In additional studies in neonatal tissue, glycine (200 μM) effectively stimulated [^{3}H]DA release from striatal slices. Although the basal efflux of [^{3}H]DA was greater in neonatal tissue than adult tissue $P < 0.05$, t test), the magnitude of glycine evoked DA release was greater in the neonatal tissue than in the adult tissue ($P < 0.01$, t test) (Fig. 6).

Results of the release experiments are plotted as efflux curves. Each point on the curve is a rate constant, which is the percentage of radioactivity in the tissue at the time of collection released into the medium per minute. Each point on the efflux curve is the mean ± S.E.M. of four or eight determinations.

DISCUSSION

Neurotransmitter release from nerve terminals is believed to be sensitive to calcium [13] and many studies have utilized calcium-dependent, potassium-evoked release of previously taken up radiolabelled transmitter to be characteristic of neurotransmitter release from nerve terminals [3–6, 14, 15]. Therefore, the demonstration of calcium-dependent, potassium-evoked release of [^{3}H]GABA, [^{3}H]DA and [^{3}H]-5-HT

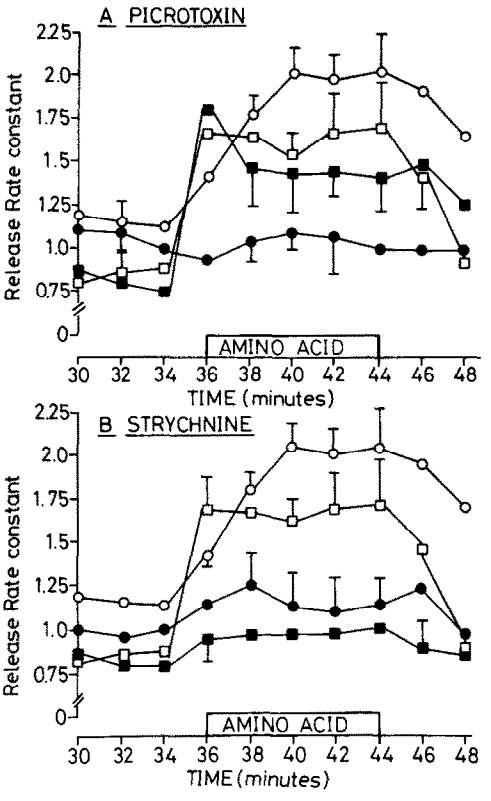

Fig. 5. A. Effect of picrotoxin on the GABA- and glycine-evoked efflux of radioactivity from superfused slices of adult rat striatum prelabelled with 10^{-8} M [^{3}H]dopamine *in vitro*. Open symbols represent control efflux of radioactivity in the presence of 400 μM GABA (○) or 400 μM glycine (□) Closed symbols represent the effect of 400 μM GABA (●) or 400 μM glycine (■) in the presence of picrotoxin (50 μM). The amino acids were added for 8 min, as indicated by the bar, in all instances; where appropriate picrotoxin was present throughout the whole superfusion period. B. Effect of strychnine on GABA- and glycine-evoked efflux of radioactivity from superfused slices of adult rat striatum prelabelled with 10^{-8} M [^{3}H]dopamine. See legend to Fig. 6A for experimental details. (○), 400 μM GABA alone; (□), 400 μM glycine alone; (●), 400 μM GABA in the presence of 0.5 μM strychnine; (■—■), 400 μM glycine in the presence of 0.5 μM strychnine.

from rat striatal slices suggests that our superfusion system is an adequate one in which to study release processes *in vitro*.

Both GABA and glycine evoked the release of [^{3}H]DA. The threshold dose of GABA was 50 μM, compared to 200 μM for glycine. This does not strictly imply that GABA is the more potent of the two, since the relative affinities of the two amino acids for avid inactivating uptake systems is not known. The stimulation of striatal DA release produced by GABA substantiates several reports [4, 5]. The ability of glycine, however, to release [^{3}H]DA is not well documented. Furthermore, the rat striatum seems to possess specific glycine receptors, since the effect was blocked by a dose of strychnine (0.5 μM) believed to be selective for glycine receptors [16], but not by 50 μM picrotoxin. This would also suggest that glycine does not exert any of its DA releasing actions through GABA receptors. The data from this study would suggest that GABA-evoked DA release is not a simple action at GABA receptors. Furthermore, while separately both amino acids evoked release of [^{3}H]DA, equi-effective doses of GABA (100 μM) and glycine (300 μM) together produced an effect which was less than additive; such a result might suggest some overlap between the effects of GABA and glycine (Kerwin and Pycock, unpublished observation). It has been demonstrated that GABA has agonist properties at glycine receptors in the spinal cord [11], and our observation that GABA-evoked DA release is blocked by a low dose of strychnine would suggest that some of the GABA-evoked DA release may be mediated at glycine receptors. However, strychnine produced a total blockade of GABA-evoked DA release; this is not compatible with the hypothesis that both GABA and glycine receptors are present to stimulate DA release. However, the ability of strychnine to dissociate receptors for ω-amino acids has been questioned by at least one group [17].

It could be suggested that GABA evokes DA release only by an action at β-alanine or taurine receptors. Taurine and β-alanine may be important inhibitory neurotransmitters [18], and sensitivity to both strychnine and picrotoxin is characteristic of the actions of taurine and β-alanine, both spinally [19] and supraspinally [20]. However, this does not seem likely in view of

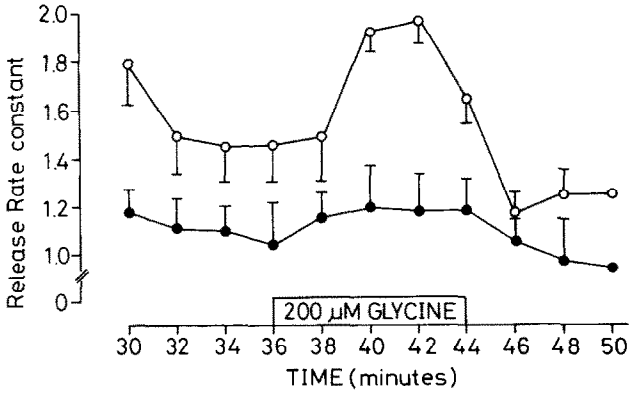

Fig. 6. Effect of 200 μM glycine on the efflux of radioactivity from superfused slices of adult (●—●) or 2-day-old (○—○) rat striatum prelabelled with [^{3}H]dopamine *in vitro*. As indicated by the bar, glycine was added for 8 min, 36 min after the start of superfusion. Results are shown as described in legend to Fig. 1.

the fact that at 500 μM neither taurine nor β-alanine stimulated [^{3}H]DA release.

[^{3}H]Glycine was released by potassium in a calcium-dependent fashion from both neonatal spinal cord and striatal slices. This is some evidence that glycine may function as a transmitter substance released from nerve terminals in the rat striatum. We were unable to demonstrate [^{3}H]glycine release from adult striatal slices. However, a similar problem was also encountered in the spinal cord where glycine is unequivocally an important neurotransmitter [11]. Similar results have been obtained for [^{3}H]GABA release from dorsal column nuclei*, where K^+ only evoked release of label from neonatal tissue. It was concluded that the glial cell system, not fully developed in the neonate, prevented the manifestation of [^{3}H]GABA release. Similarly, in this study the glial cell system may be preventing released [^{3}H]glycine from appearing in the superfusate by avidly taking up released amino acid [21].

In support of this idea, 200 μM glycine was more potent at releasing [^{3}H]DA from neonatal slices than from adult slices, the postulated uptake process preventing exogenous glycine from reaching the tissue receptors. Furthermore, in these experiments the spontaneous efflux of [^{3}H]DA was significantly greater in the neonate tissue, suggesting that DA release may be subject to similar influences.

It is possible that DA release may be regulated at the nerve terminal by extradopaminergic influences. One such controlling influence may be the release of glycine and GABA onto dopamine terminals in the striatum.

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* R. G. Hill, F. Roberts and J. F. Mitchell, personal communication.

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