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REVERSIBLE EFFECTS OF TETANUS TOXIN ON STRIATAL-EVOKED RESPONSES AND [^{3}H]-γ-AMINOBUTYRIC ACID RELEASE IN THE RAT SUBSTANTIA NIGRA

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1 The effects of sublethal doses of tetanus toxin on γ-aminobutyric acid (GABA)-mediated synaptic transmission and [^{3}H]-GABA release were studied in the rat substantia nigra.

2 Intranigral injections of tetanus toxin at 1–5 times the mouse LD_{50} dose produced ipsiversive circling behaviour which was maximal after 1 week and lasted 2–3 weeks. Rats then displayed normal behaviour suggesting that the effects of the toxin were fully reversible.

3 In the treated nigra of circling rats there was a reduction in the striatal-evoked inhibition of compacta and reticulata neurones, but no change in their spontaneous firing rates. Some forms of striatal-evoked excitation were also reduced. Once rats had recovered from circling no alterations in the synaptic responses were detected.

4 In circling rats there were no differences in the sensitivities of neurones in the treated and untreated nigra to GABA or to other inhibitory neurotransmitters.

5 The Ca^{2+}-dependent, K^+-evoked release of [^{3}H]-GABA from slices prepared from the treated nigra of circling rats was less than that from the untreated nigra of circling rats. No differences in nigral [^{3}H]-GABA release were observed once rats had recovered from the circling behaviour.

6 The results demonstrate that doses of tetanus toxin which produce reversible behavioural effects can interfere reversibly with GABA-mediated synaptic transmission by a presynaptic mechanism which probably involves a reduction in transmitter release.

Introduction

The pathogenic action of tetanus toxin is generally believed to be predominantly due to interference with inhibitory synaptic transmission in the central nervous system (Mellanby & Green, 1981). In particular, the toxin has been shown to block presynaptically γ-aminobutyric acid-(GABA) and glycine-mediated synaptic inhibition in the spinal cord and at higher centres (Curtis & DeGroat, 1968; Guschin, Kozhechkin & Sverdlov, 1969; Davies & Tongroach, 1979) probably by reducing the release of these inhibitory amino acid transmitters (Osborne, Bradford & Jones, 1973; Collingridge, Collins, Davies, James, Neal & Tongroach, 1980a; Collingridge & Davies, 1980a; Collingridge, Thompson, Davies & Mellanby, 1981; Bigalke, Heller, Bizzini & Habermann, 1981). However, in these studies extremely high doses of tetanus toxin were used in order to produce rapid effects suitable for acute studies. It is therefore possible that the effects observed were artefacts caused by the high toxin concentration, as has been suggested for studies with the closely related botulinum toxin (Hagenah, Benecke & Wiegand, 1977). We have, therefore, investigated the action of much lower, sublethal doses of tetanus toxin on synaptic transmission and transmitter release in the central nervous system.

Experiments were performed in the rat substantia nigra since this structure receives a massive GABAergic projection from the striatum (Dray, Gonye & Oakley, 1976; Collingridge & Davies, 1981) which is sensitive to tetanus toxin, when applied locally in high doses (Davies & Tongroach, 1979; Collingridge & Davies, 1981). Furthermore, high doses of the toxin also reduce [^{3}H]-GABA release from nigral slices (Collingridge *et al.*, 1980a; Collingridge & Davies, 1980a).

Some of these results have appeared in preliminary form (Collingridge & Davies, 1979a; 1980b).

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Methods

Injection of tetanus toxin

Female rats weighing 200–300 g were injected with 1–5 times the LD_{50} of tetanus toxin for mice (in 0.5 μl physiological saline containing 0.2% gelatine) into one substantia nigra, by means of a Hamilton syringe and while under halothane (1–1.5%) anaesthesia. See Collingridge *et al.*, 1980a; Collingridge & Davies, 1980b for details of injection procedures and measurement of behavioural changes.

Electrophysiology

The electrophysiological techniques have been described in detail previously (Collingridge & Davies, 1979b). Briefly, extracellular recordings were obtained, using glass microelectrodes, from single nigral neurones in rats anaesthetized with halothane (1.0–1.5%) before and various times after an intranigral injection of tetanus toxin. Often recordings were obtained from the same animal on more than one occasion. The ipsilateral striatum was stimulated with 0.3 ms pulses delivered at 0.5–2.0 Hz, and the stimulus current (20–300 μA) necessary to evoke responses measured. Higher stimulus intensities were sometimes required (up to 3000 μA) to antidromically activate neurones. In some experiments the following drugs were administered by current balanced iontophoresis from a 7-barrelled electrode (tip diameter 7–15 μm): γ-aminobutyric acid (GABA; 0.5 M, pH 3.5), glycine (0.5 M, pH 3.5), dopamine hydrochloride (0.5 M, pH 4.0), noradrenaline bitartrate (NA; 0.5 M, pH 4.0), 5-hydroxytryptamine bimaleinate (5-HT; 0.25 M, pH 4.0), bicuculline methochloride (BMC; 0.005 M in 0.165 M NaCl, pH 3.5) and strychnine sulphate (0.005 M in 0.165 M NaCl, pH 7.0). In these experiments the recording electrode was cemented to (but projected 15–30 μm in front of) the iontophoretic electrode. Nigral neurones were identified by their characteristic electrophysiological properties (Collingridge & Davies, 1981) and at the end of the final recording session in each animal the last recording and stimulation sites were marked and determined histologically (Collingridge & Davies, 1979b).

Release studies

Various times after an intranigral injection of tetanus toxin, rats were killed and slices prepared for neurochemical studies. The spontaneous and K^+-evoked release of exogenously accumulated [^{3}H]-GABA from the treated and untreated contralateral nigra were compared, as described in detail previously (Collingridge *et al.*, 1980a). Briefly, 0.35 mm thick slices were incubated for 40 min in 10 ml oxygenated Krebs-Ringer bicarbonate solution at 37°C, the last 30 min in the presence of [^{3}H]-GABA (2.9×10^{-9} M). Slices were then transferred to small chambers and superfused with medium at room temperature. After 30 min, 2 min fractions were collected and the radioactivity in these and in the slices at the end of the experiment were determined by means of liquid scintillation spectrometry. Release was evoked by exchanging the superfusion medium with one containing an elevated KCl concentration (50 mM) for 2 min. The radioactivity in each fraction, corrected for efficiency of counting, was expressed as the percentage of the amount present in the slices at that time. Evoked release was calculated as the total increase above the spontaneous release; the latter being determined from the 6 min period preceding superfusion with a high KCl-containing medium. Amino-oxyacetic acid (0.1 mM) was present throughout to prevent metabolism of GABA and under these conditions it has been shown that approximately 97% of released tritium is in the form of [^{3}H]-GABA (Srinivasan, Neal & Mitchell, 1969).

Tetanus toxin

The preparation, assay and neutralization, with tetanus antitoxin, of the toxin have been described elsewhere (Collingridge *et al.*, 1980a).

Results

Behavioural effects

Unilateral intranigral injections of active, but not neutralized, tetanus toxin (1–5 × mouse LD_{50}) produced ipsiversive circling behaviour. Upon recovery from anaesthesia (5–10 min after the injection) no obvious behavioural changes were observed and circling behaviour was not detected until 1–2 days after 4–5 × mouse LD_{50} or 3–6 days after 1–2 × mouse LD_{50}. The peak effect was observed 6–10 days after the injection and then progressively subsided over the next 3–10 days (see Figure 1 in Collingridge & Davies, 1980b). Effects then appeared to have been fully reversible as assessed by the animal's gross behaviour.

Initially, rats only circled ipsiversively in response to a physical stimulus but as time progressed they also circled spontaneously when placed in a novel environment (Figure 1). During the peak effects of the higher doses, rats circled in response to very weak physical stimuli, loud auditory stimuli and sometimes visually presented stimuli but their spontaneous circling in a novel environment declined. In a few of the most strongly affected rats, and in those injected with

more than 5 times mouse LD_{50} of toxin, circling was progressively replaced by ipsiversive barrel-rolling (rotation directed about the longitudinal body axis) and 1–2 days later by death.

Circling rarely involved any postural asymmetry and appeared as a rapid swivelling motion around the axis of the ipsilateral hind limb which often encompassed approximately 180° or 360° at a time. It is therefore very distinct from the robust nose-to-tail circling seen with, for example, intranigral GABA agonists (cf Kilpatrick & Starr, 1981). No other obvious behavioural effects were detected.

Electrophysiological effects

Results were obtained from two types of cell in the substantia nigra, distinguished by their electrophysiological properties (Collingridge & Davies, 1981) and their location in the nigra, as determined from histological verification of electrode placements. The first type was located in the pars reticulata, had narrow action potentials, usually had high spontaneous firing rates (10–50 spikes/s) and was rarely antidromically activated following striatal stimulation. The second type was predominantly found in the pars compacta, had wide action potentials, low spontaneous firing rates (below 9 spikes/s) and was often antidromically activated by striatal stimulation, in a characteristic manner (Collingridge, James & MacLeod, 1980b). These two cell types were also distinguishable by their orthodromic responses to striatal stimulation and by their responses to iontophoretically administered drugs (Collingridge & Davies, 1981).

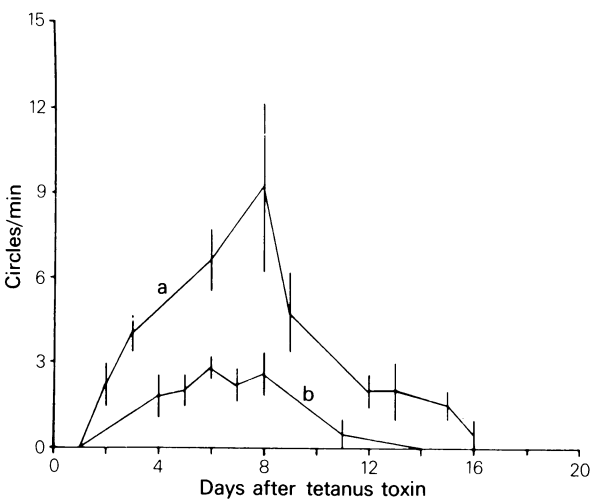

Figure 1 Spontaneous ipsiversive circling behaviour produced by intranigral injections of tetanus toxin. Complete (360°) circles were scored for 1 min on placing the rat in a novel environment. Each point is the mean circles/min of 5 rats which received 5 (a) or 3 (b) mouse LD_{50} of tetanus toxin; vertical lines indicate s.e.mean.

Before an intranigral injection of tetanus toxin (control) reticulata neurones responded to striatal stimulation with mixed periods of excitation and inhibition. When similar recordings were obtained from these and other rats 6–13 days following an intranigral injection of tetanus toxin (while the rats were circling ipsiversively) fewer reticulata neurones appeared to be inhibited by striatal stimulation in the injected nigra (Table 1). There was also an increase in the stimulus current necessary to produce the early inhibitory period (Table 1). In addition, fewer neurones responded with a period of late excitation although a similar number displayed early excitatory periods (Table 1). Despite these changes in synaptic responses, toxin treatment had no effect on the spontaneous firing rate of reticulata neurones (Table 1). The toxin-induced reductions in inhibition and excitation were reversible since there were no differences between control and toxin-injected rats once the latter had recovered from the circling behaviour (recovery rats, Table 1). Examples of the striatal evoked responses of reticulata neurones from the same rat before, during and after toxin-induced turning are illustrated in Figure 2. In some experiments the spontaneous firing rate and responses of reticulata neurones to striatal stimulation were measured in the untreated nigra of circling rats and found to be similar to controls. Finally, injections of neutralized tetanus toxin did not affect striatal-evoked inhibition of reticulata neurones, recorded appproximately 1 week later (Collingridge & Davies, 1980b).

The response of compacta neurones to striatal stimulation in controls also consisted of mixed periods of excitation and inhibition. In the treated nigra of circling rats, but not in the untreated nigra, the treated nigra of recovery rats or the nigra injected with neutralized toxin, there were fewer compacta neurones inhibited by striatal stimulation and the duration of inhibition was shorter (Table 2). In addition, the incidence of late excitation, which followed the initial long lasting inhibition, was also reduced in circling rats (Table 2). The effect of the toxin on the early excitatory response was unclear (Table 2). Toxin treatment had no effect on the spontaneous firing rate of compacta neurones or on their antidromic invasion following striatal stimulation (Table 2).

The responses of reticulata and compacta neurones to iontophoretically administered neurotransmitters in the treated and untreated nigra of circling rats were similar. Thus, in both cases, GABA and glycine inhibited all neurones, 5-HT often inhibited reticulata neurones and NA and dopamine usually inhibited compacta neurones (Table 3). Ex-

Table 1 Effect of tetanus toxin on the response of reticulata neurones to striatal stimulation

Group	No. of cells	% cells responding with:				Threshold for inhib (μA)	Firing rate (spikes/s)
		early excit	early inhib	late excit	late inhib		
a	35	26	87	74	40	65.6± 6.9	22.3±2.1
b	73	27	64	36	16	139.1±11.1*	22.9±1.8
c	30	30	83	70	40	72.7±10.3	28.0±2.3

Four components of the response to 300 μA striatal stimulation were analysed. Early excitation (latency usually 2–6 ms) was the initial response when present. Early inhibition (latency less than 15 ms) followed this excitation or was the initial response. Late excitation was the response which often followed the inhibition and was itself sometimes followed by further (late) inhibition (see Collingridge & Davies, 1981 for further details). The following treatment groups were compared: (a) Control; (b) circling rats (recordings obtained from the injected nigra 6–13 days after toxin); and (c) recovery rats (recordings obtained 3 days–6 months after circling had subsided). Results were obtained from 3–13 rats per group and some of the data (and additional data) were used to construct Table 1 in a preliminary report (Collingridge & Davies, 1980b).

*Significance of difference of threshold for early inhibition (two tailed *t* test): $P < 0.001$ (Group b compared to group a or c).

Table 2 Effect of tetanus toxin on the response of compacta neurones to striatal stimulation

Group	No. of cells	% cells responding with:				A.D. Spike	Duration of inhib (ms)	Firing rate (spikes/s)
		early excit	early inhib	late excit	late inhib			
a	10(9)	55	88	44	22	40	127±17	4.6±0.6
b	24(18)	17	56	28	0	33	60±13*	4.9±0.3
c	23(8)	25	100	50	38	48	105±31	5.2±0.3
d	17(9)	55	100	44	22	27	126±36	4.8±0.4
e	9(6)	17	83	50	17	11	119±21	5.5±0.7

Four components of the orthodromic response to 300 μA striatal stimulation were analysed: early excitation and inhibition occurred with latencies of less than 30 ms, late excitation followed the early inhibition and was itself often followed by late inhibition (see Collingridge & Davies, 1981 for further details). The no. of cells tested for antidromic invasion and used to determine firing rates are given; of these only the number in parentheses were used to evaluate the orthodromic responses. The following treatment groups were compared: (A) Control; (b) circling rats (recordings obtained from the injected nigra 6–13 days after toxin); (c) circling rats (recordings obtained at the same time as in (b) from the contralateral nigra); (d) recovery rats (recordings from the injected nigra made 3 days–6 months after circling had subsided); (e) neutralized toxin-injected rats (recordings made from the injected nigra after 6–13 days). Data were obtained from 3–13 rats per group.

*Significance of difference of inhibitory duration compared to control (two tailed *t* test): $P < 0.02$.

Table 3 Effect of tetanus toxin on responses of nigral neurones to iontophoretically applied inhibitory neurotransmitters

Transmitter	Compacta						Reticulata					
	Control			Toxin			Control			Toxin		
	+	0	−	+	0	−	+	0	−	+	0	−
GABA	0	0	8	0	0	8	0	0	16	0	0	15
Glycine	0	0	5	0	0	4	0	0	12	0	0	11
Dopamine	0	3	2	0	0	5	1	3	1	0	4	1
5-HT	0	7	0	0	3	0	0	2	6	1	1	3
NA	0	2	3	0	0	4	0	3	0	0	3	0

The responses of neurones in the treated nigra (toxin) and untreated nigra (control) of circling rats was compared using alternate tracks with the same electrodes. The figure in each column refers to the number of cells excited (+), inhibited (−) or unaffected (0) by compounds, expelled with ejection currents of up to 200 nA

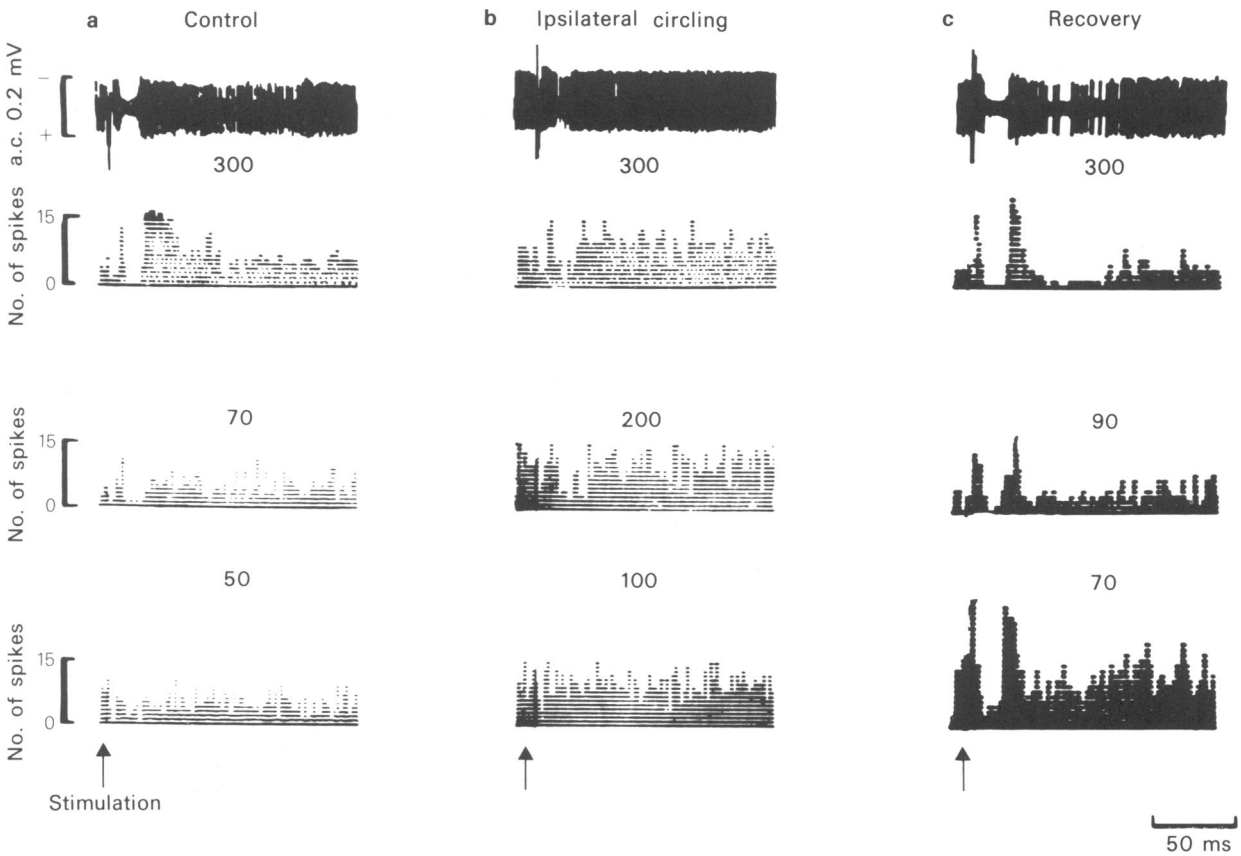

Figure 2 Effect of tetanus toxin on the striatal evoked response of reticulata neurones from the same rat. Recordings were obtained (a) before the toxin injection (control), (b) 6 days after an intranigral injection of tetanus toxin (when this rat was strongly turning ipsiversively) and (c) 172 days after the animal had recovered from the circling behaviour. The uppermost record in each column is a superimposed oscilloscope record of the response of a neurone to striatal stimulation (300 μA). The PSTHs immediately below were constructed at the same time. The lower two PSTHs using lower intensity stimuli (e.g. 70 and 50 μA in (a)). All records were constructed from 128 consecutive stimulus presentations (except top 3 records in (c) constructed from 64 consecutive stimuli), and PSTHs were analysed in 2 ms intervals. The position of the stimulus is indicated by the arrow and the stimulus artefact is present in most records. The neurone in column (a) shows a typical short inhibitory period followed by a period of excitation. There is also a brief period of excitation preceding the inhibition. The inhibition is evoked by 70 μA but not by 50 μA stimulating current intensity. Cell (b) shows simple inhibition but this inhibition is weak and only observed when high stimulus currents were used. Cell (c) shows an excitatory-inhibitory-excitatory sequence followed by a further period of inhibition. The initial inhibition is still present at a stimulus intensity of 70 μA. (Spikes retouched).

amples of the response of a compacta neurone to these agents in the treated nigra of a circling rat are shown in Figure 3. Similar expelling currents of GABA were required to produce approximately equivalent depressions of neuronal firing in treated compared to untreated nigra (in both cases reticulata neurones were the more sensitive cell type to this inhibitory amino acid). Furthermore, the median ratio of ejecting currents required to depress similarly neurones in the treated compared to untreated nigra calculated for 9 electrodes was 1.0, indicating no change in GABA sensitivity. The sensitivities of both reticulata and compacta neurones to glycine were also similar in treated compared to untreated nigra. The responses of both cell types to GABA and glycine in the treated nigra could be selectively antagonized by bicuculline methochloride and strychnine, respectively (Figure 4).

The residual striatal-evoked inhibition of neurones remaining in the toxin-injected nigra of circling rats was further reduced by bicuculline methochloride (3 reticulata and 1 compacta cells) but not by strychnine (2 reticulata cells; Figure 4).

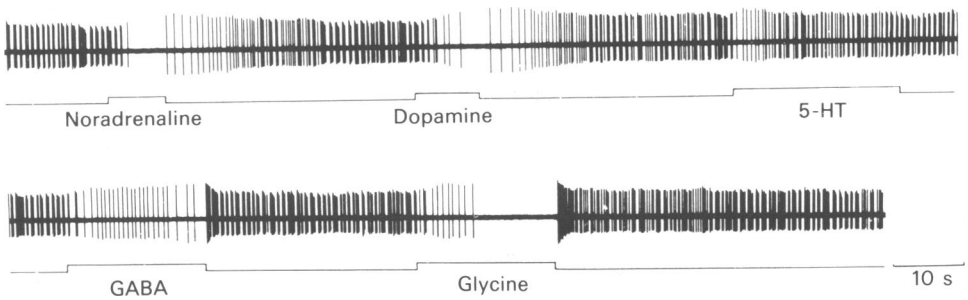

Figure 3 Response of a compacta neurone in the injected nigra of a circling rat to inhibitory neurotransmitters. A continuous moving film record (retouched) shows that all drugs except 5-hydroxytryptamine (5-HT) inhibited this cell and that inhibition was accompanied by an increase in spike height. All compounds were applied using 100 nA expelling current for the time shown by the upward deflections in the trace below the spike records.

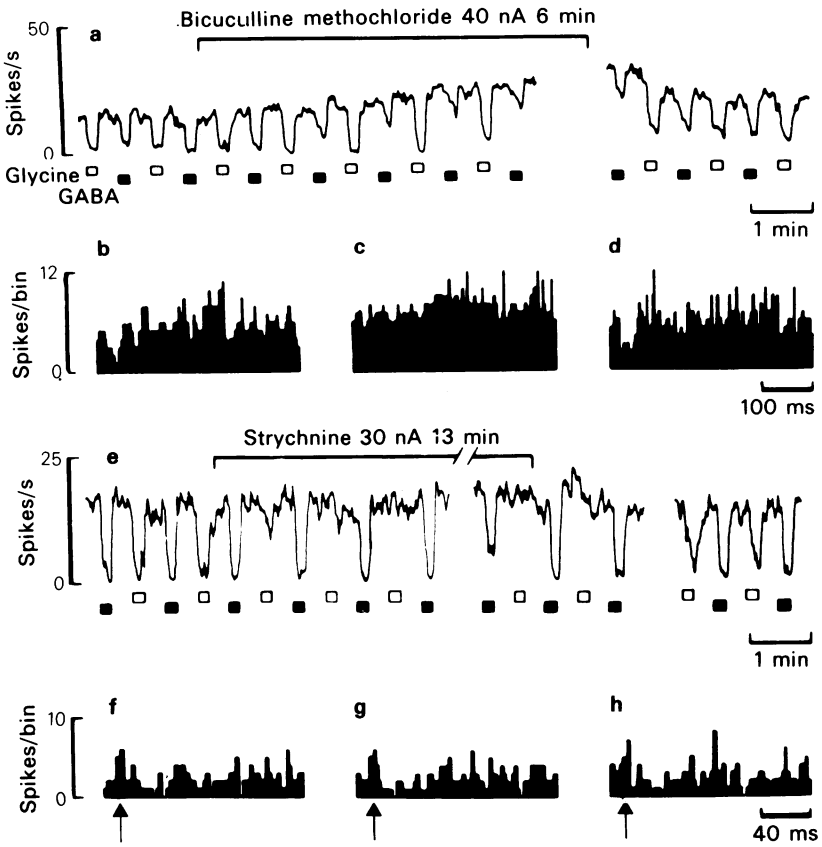

Figure 4 Effects of bicuculline methochloride and strychnine on a reticulata neurone in the toxin-treated nigra of a circling rat. (a) Ratemeter record illustrating antagonism by bicuculline methochloride (BMC) of γ-aminobutyric acid-(GABA, 15 nA), but not glycine (50 nA)-induced depression. Note the increase in spontaneous firing rate produced by BMC. (b) Control PSTH (128 sweeps, 2 ms bins) showing weak inhibition of this cell evoked by striatal stimulation (300 μA). (c) PSTH consisting of 74 sweeps from the same neurone showing antagonism of the inhibition by BMC (40 nA for 5 min, constructed at the same time as the break in the ratemeter record). (d) Recovery of the evoked inhibition 3 min after terminating the BMC ejection. (e) Ratemeter record illustrating selective antagonism of glycine-induced depression by strychnine. PSTHs in (f)–(h) (64 sweeps, 2 ms bins) were constructed before (f), 9 min after commencing (g) and 6 min after terminating the strychnine ejection (h). Strychnine had no effect on the striatal-evoked inhibition.

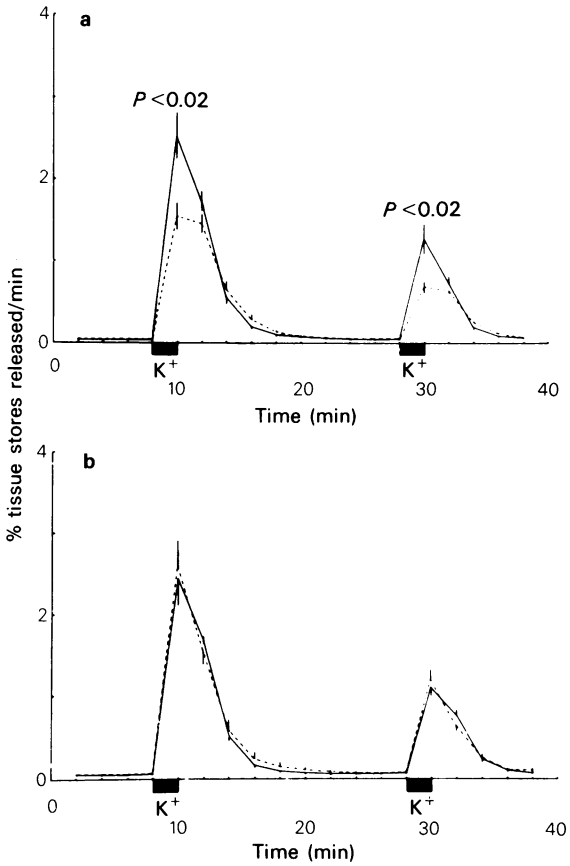

Figure 5 Comparison of the effects of tetanus toxin on spontaneous and 50 mM K^+-evoked release of [^{3}H]-γ-aminobutyric acid from slices prepared from control (continuous line) and treated (dashed line) nigra of circling (a) or recovery (b) rats. Each point is the mean release from 6 experiments; vertical lines show s.e.mean. Significant reductions of K^+-evoked release were seen only from slices prepared from circling rats.

Effects on release

Superfusion for 2 min with Krebs solution containing increased KCl (50 mM) produced a large, highly Ca^{2+}-dependent increase in tritium efflux from nigral slices. The tritium released can be assumed to be essentially all in the form of [^{3}H]-GABA (Srinivasan *et al.*, 1969). This release from nigral slices prepared from the toxin-injected and untreated, contralateral nigra of circling rats, 5–9 days after the injection, was compared in 6 paired experiments. Toxin pretreatment had no effect on resting efflux (0.038 ± 0.007% and 0.039 ± 0.004% tissue stores/min, respectively) but produced a significant reduction in the K^+-evoked efflux (21.7 ± 4.9% and 25.4 ± 6.1% reductions in response to the first and second exposures to high KCl containing medium, respectively; Figure 5). However, the small Ca^{2+}-insensitive K^+-evoked release was not reduced by tetanus toxin. There was no significant difference in either spontaneous or evoked [^{3}H]-GABA efflux from slices prepared from treated or untreated nigra of rats after they had recovered from the circling behaviour (Figure 5), suggesting that the toxin-induced changes in release were reversible.

Discussion

The present results demonstrate that a local injection of a non-lethal dose of tetanus toxin into the rat substantia nigra reduces GABA-mediated synaptic inhibition in this structure in a reversible manner. Furthermore, the action appears to be presynaptic and probably via an effect on transmitter release. A similar mode of action of tetanus toxin on both GABA- and glycine-mediated synaptic inhibition and transmitter release was suggested from studies in which much higher doses of the toxin were used (Brooks, Curtis & Eccles, 1957; Curtis & DeGroat, 1968; Guschin *et al.*, 1969; Osborne, Bradford & Jones, 1973; Davies & Tongroach, 1979; Collingridge & Davies, 1980a; Collingridge *et al.*, 1980a; Collingridge & Davies, 1981; Collingridge *et al.*, 1981). In two previous studies, investigating the central actions of low doses of tetanus toxin, it was shown that intracortical injections produced convulsions, associated changes in the electrocorticogram and a loss of cortical inhibition after a latency of a few days (Carrea & Lanari, 1962; Brooks & Asanuma, 1965); results consistent with the present findings.

The predominant electrophysiologically detectable change in the toxin-injected nigra of circling rats was a reduction in striatal-evoked inhibition of both reticulata and compacta neurones. This inhibition of both cell types is sensitive to bicuculline methochloride (Collingridge & Davies, 1981) and is, therefore, probably GABA-mediated. There is considerable anatomical and neurochemical evidence for a GABAergic pathway originating in the striatum and terminating on nigral compacta and reticulata neurones (see Dray, 1979). The inhibition remaining in the toxin-injected nigra was also probably GABA-mediated since it was further reduced by doses of bicuculline methochloride which selectively antagonized GABA. Thus, either some GABA-mediated inhibition in the nigra is resistant to tetanus toxin or the doses used were insufficient to abolish synaptic inhibition completely. This latter possibility is supported by the observation that toxin-pretreatment reduced GABA release by only 20–25% although

over 50% of nigral GABA release is toxin-sensitive (Collingridge & Davies, 1980a).

It is likely that in the nigra the release of exogenously accumulated [^{3}H]-GABA is from nerve terminals (Collingridge *et al.*, 1980a) and that tetanus toxin affects this release by a direct action on these structures (Collingridge & Davies, 1982). Since in these studies (Collingridge *et al.*, 1980a, Collingridge & Davies, 1982) effects on release were produced without any alteration in [^{3}H]-GABA uptake or GABA levels it was concluded that the toxin probably acted directly on the release process. Accordingly, in the present study only effects on [^{3}H]-GABA release were investigated. An additional action of tetanus toxin on nigral GABA uptake under the present conditions is unlikely since the sensitivity of neurones to iontophoretically administered GABA was unaltered. Confirmation that the action of tetanus toxin is directly on release mechanisms requires that its effects be examined on endogenous transmitter release evoked by specific stimulation of neuronal pathways.

In addition to affecting synaptic inhibition, tetanus toxin also reduced some types of striatal-evoked excitation of nigral neurones. These effects of the toxin may have been due to a direct action on excitatory transmission, a secondary effect caused by the reduction in inhibition or a compensatory mechanism. In the case of the long latency excitation of compacta neurones, a secondary effect seems likely since this excitation decreases in size when the preceding inhibition is reduced by bicuculline methochloride (Collingridge & Davies, 1981). With reticulata neurones the short latency striatal-evoked excitations appeared unaffected by the toxin-treatment, supporting the acute observations with higher doses (Davies & Tongroach, 1979). Although it is not known to what extent other systems are affected in the nigra, the effects on striatal-evoked responses are nevertheless consistent with the notion that tetanus toxin primarily affects synaptic inhibition. However, there were no associated increases in the spontaneous firing rate of nigral neurones suggesting that either compensatory changes had occurred or possibly that the transmitter systems affected in the nigral region were not tonically active. The increase in firing rate produced by bicuculline methochloride (eg. Figure 5) was not inconsistent with this latter possibility since this substance has an excitatory action independent of its GABA receptor antagonism (Hill, Simmonds & Straughan, 1976).

The ipsiversive circling behaviour described here is consistent with a reduction in nigral GABA release since similar circling is seen following intranigral isoniazid (Gale & Iadarola, 1980), a substance that reduces GABA release indirectly by inhibiting its synthesis, and by postsynaptic GABA antagonists (see Pycock, 1980). Intranigral injections of much higher doses of tetanus toxin also predominantly produce ipsiversive circling behaviour (James & Collingridge, 1979; McGeer, McGeer & Campbell, 1980; Collingridge *et al.*, 1980a) although dopamine-dependent contraversive circling is observed with injections into the rostral nigra (James & Collingridge, 1979). The contraversive circling may be due to disinhibition of nigrostriatal dopaminergic neurones whereas the ipsiversive circling probably results from removal of inhibitory influences onto other nigral output pathways, in particular nigrothalamic and nigrotectal systems (Di Chiara, Porceddu, Morelli, Mulas & Gessa, 1978; Kilpatrick, Starr, Fletcher, James & MacLeod, 1980; Imperato, Porceddu, Morelli, Faa & Di Chiara, 1981; Kilpatrick, Collingridge & Starr, 1982).

In conclusion, since the nigra has an exceedingly large GABAergic innervation, which is mainly via a long pathway from the striatum, it would appear an ideal system in which to investigate the action of tetanus toxin on central GABA systems. In the present study the time course and total reversibility of the behavioural changes resemble the condition in non-lethal cases of tetanus. It is therefore likely that the nigral toxin concentrations were similar to those seen in various parts of the central nervous system in the pathological condition. Consequently, it is likely that in tetanus there is a reduction in GABAergic inhibition due to impairment of the release of this substance. Further studies are required to determine which other transmitter systems are sensitive to pathogenically relevant doses of tetanus toxin, and to discover which regions of the central nervous system, in addition to the spinal cord, are affected in experimental tetanus.

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