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TETANUS TOXIN AND SYNAPTIC INHIBITION IN THE SUBSTANTIA NIGRA AND STRIATUM OF THE RAT

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SUMMARY

1. The effects of tetanus toxin were determined on GABA-mediated synaptic inhibition of substantia nigra neurones evoked by striatal stimulation and on the presumed dopamine- and 5-hydroxytryptamine-mediated synaptic inhibition of striatal neurones evoked by nigral and dorsal raphe nucleus stimulation, respectively, in the urethane-anaesthetized rat.

2. Following an intranigral injection of tetanus toxin, striatal-evoked inhibition of substantia nigra neurones, which is sensitive to bicuculline, was rapidly abolished. This effect was not accompanied by any significant change in the responses of nigral neurones to ionophoretically administered GABA or other putative neurotransmitters and thus indicates a presynaptic site of action of the toxin.

3. The rate of onset of action of the toxin in the substantia nigra was extremely rapid (1–4 min) and appeared to be related to the rate of activation of the inhibitory pathway.

4. Injections into the substantia nigra of tetanus toxin neutralized with antitoxin had no significant effect on striatal-evoked inhibition in the substantia nigra.

5. Injections of tetanus toxin into the striatum failed to influence the inhibition of striatal neurones evoked by stimulation of the ipsilateral substantia nigra or the dorsal raphe nucleus, suggesting that tetanus toxin does not impair monoamine-mediated inhibition in the central nervous system.

6. Synaptic excitation which preceded substantia-nigra-evoked inhibition in striatal neurones and which occasionally preceded striatal-evoked inhibition in nigral neurones was also unaffected by tetanus toxin.

7. It is suggested that tetanus toxin selectively abolishes GABA-mediated synaptic inhibition in the central nervous system and may be a useful tool in the identification of such synaptic inhibitory mechanisms.

INTRODUCTION

It has been proposed that tetanus toxin might be a useful neurobiological agent for producing a long lasting impairment of inhibitory synaptic transmission in the

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mammalian central nervous system (Curtis, 1971; Mellanby, George, Robinson & Thompson, 1977). These proposals were based mainly on observations made on spinal neurones that tetanus toxin abolishes strychnine-sensitive and bicuculline-sensitive synaptic inhibition by interfering with the release of the inhibitory neurotransmitter substances glycine or GABA from nerve terminals (Curtis & De Groat, 1968; Curtis, Felix, Game & McCulloch, 1973). However, it is not clear whether tetanus toxin has similar effects on supraspinal neurones to those reported on spinal neurones. Consistent with its actions on spinal inhibition tetanus toxin has been reported to interfere with the inhibitory effects of basket cells on cerebellar Purkinje neurones (Curtis *et al.* 1973) and the recurrent inhibition of cerebral cortical neurones evoked by medullary stimulation (Brooks & Asanuma, 1965). Both these supraspinal inhibitions are sensitive to bicuculline and are therefore probably mediated by GABA (Curtis & Felix, 1971). However, two other supraspinal inhibitions, which subsequent studies indicated were also sensitive to bicuculline (Curtis, Duggan & Felix, 1970; Curtis & Felix, 1971), were unaffected by tetanus toxin. These were the inhibitory effects of cerebellar Purkinje neurones on Deiters' nucleus (Obata, Ito, Ochi & Sato, 1967), and the inhibition of cerebral cortical neurones evoked by stimulation of the cortical surface (Krnjević, Randic & Straughan, 1966). It therefore seemed worthwhile establishing the effects of tetanus toxin on a long supraspinal inhibitory pathway which utilizes GABA as a transmitter. To this end an examination was made of the effects of tetanus toxin on the inhibitory effect of striatal stimulation on neurones in the substantia nigra. Electrophysiological and neurochemical data indicates that this pathway is relatively long and that the inhibitory effect of striatal stimulation is mediated by GABA or a GABA-like substance (Frigyesi & Purpura, 1967; Feltz, 1971; Precht & Yoshida, 1971; Crossman, Walker & Woodruff, 1973; Fonnum, Grofova, Rinvik, Storm-Mathisen & Walberg, 1974; Dray, Gonye & Oakley, 1976).

An important feature of tetanus toxin as a physiological tool would be its selectivity for amino acid mediated synaptic events as opposed to inhibition mediated by other transmitter substances. To test this hypothesis we have examined, in concurrent experiments, the effects of the toxin on presumed monoamine-mediated inhibition evoked in the striatum by both substantia nigra and dorsal raphe nucleus stimulation. Previous electrophysiological studies suggest that these synaptic events are mediated by dopamine and 5-hydroxytryptamine respectively (Bloom, Costa & Salmoiraghi, 1965; McLennan & York, 1967; Connor, 1970; Feltz & de Champlain, 1972; Gonzales-Vegas, 1974; Liles, 1974; Miller, Richardson, Fibiger & McLennan, 1975; Zarzecki, Blake & Somjen, 1976; Olpe & Koella, 1977; Davies & Tongroach, 1978). The results obtained indicate that tetanus toxin selectively abolishes amino-acid-mediated synaptic inhibition in the central nervous system.

Preliminary reports of some of these results have already been published (Davies & Tongroach, 1977; Straughan, Davies & Tongroach, 1978).

METHODS

Experiments were performed on adult male rats anaesthetized with urethane (1·2–1·4 g.kg^{-1}).

General procedures

Substantia nigra experiments. Stainless steel bipolar stimulating electrodes (tip separation 0·25 mm, insulated to within 0·25 mm of the tip) were positioned stereotaxically in one striatum (L1·5–4·0; A6·0–9·5; D2·0 to −1·8; Konig & Klippel, 1963) and single unit activity was recorded from the ipsilateral substantia nigra by means of the centre barrel filled with 4 M NaCl of a seven barrel micro-electrode assembly. The outer barrels of the electrode contained various combinations of the substances listed below to be ejected using standard micro-electrophoretic techniques.

Striatal experiments. In experiments on the striatum, stimulating electrodes were positioned stereotaxically in the ipsilateral substantia nigra (L2·0–3·0; A1·0–3·0; D0·8–2·6; Konig & Klippel, 1963) and dorsal raphe nucleus (A0·35; L0; D1·0; Konig Klippel, 1963) and unit activity was recorded from single striatal neurones using similar multibarrel electrodes to those used for recordings from the neurones of the substantia nigra.

Substances administered by micro-iontophoresis. These were DL-homocysteate (DLH, 0·2 M, pH 7·2), GABA (0·5 M, pH 3·5), glycine (0·5 M, pH 3·5), bicuculline methochloride (BMC, 0·005 M in 0·165 M NaCl), strychnine HCl (0·005 M in 0·165 M NaCl), dopamine HCl (0·5 M, pH 4), 5-hydroxytryptamine bimaleinate (5-HT, 0·5 M pH 4) and pontamine sky blue (0·2% in 2 M sodium acetate).

Stimulating parameters. These were single rectangular pulses 20–100 μA, duration 300 μsec at 2 Hz for striatal stimulation and 50–200 μA, duration 100–300 μsec at 0·5–1 Hz for substantia nigra and dorsal raphe nucleus stimulation. The stimulus intensities used were in the range 1·5–2 times the threshold intensity necessary to evoke responses in target neurones.

Recording of neuronal activity. Action potentials were amplified, electronically counted and displayed using conventional techniques. On line peristimulus histograms were computed (PDP-12 or Biomac 1000) to analyse stimulus-evoked inhibition in target neurones.

Location of stimulation and recording sites. All stimulation and recording sites were verified histologically after termination of the experiments and fixation of the brain (see Davies & Tongroach, 1978).

Tetanus toxin

Toxin preparation. 1 mg freeze-dried tetanus toxin (Burroughs Wellcome) containing 10^6 mouse LD50 doses/mg (L + 72 units/mg) was dissolved in 1 ml. phosphate buffer (pH 7·4), purified by dialysis against phosphate buffer (pH 7·4) and stored at 4 °C until required for use. The toxicity of the dialysed toxin was determined regularly by bioassay in groups of mice (see Mellanby & Thompson, 1972).

Micro-injection of tetanus toxin. Tetanus toxin micro-injections were made from a separate glass micropipette (tip diameter 15–30 μm), either placed stereotaxically 0·2–1 mm from or cemented to the recording pipette such that the latter protruded 500–800 μm beyond the toxin pipette. In either case 0·5–1 μl of toxin was injected by means of an Agla micrometer syringe attached to the toxin pipette via rigid polythene tubing. The effects of the toxin on central neurones were similar, regardless of the type of micropipette from which it was administered.

Neutralization of tetanus toxin. Tetanus toxin was neutralized by incubating it for 1 hr at room temperature with an excess of tetanus antitoxin (Burroughs Wellcome). Following this procedure, injections of up to 100 times the original LD_{50} dose of toxin into mice failed to produce clinical signs of tetanus.

Effects of tetanus toxin on substantia nigra neurones

The effects of tetanus toxin on striatal-evoked and ionophoretic drug-evoked responses in spontaneously firing substantia nigra neurones were determined following either 'acute' or 'chronic' intranigral injections of the toxin.

'Acute' experiments. In these studies control responses to ionophoretic administration of a number of putative neurotransmitter substances were first recorded, then a series of ten to fifteen peristimulus histograms of striatal-evoked responses was constructed. Once the reproducibility and characteristics of these responses had been established, the toxin was injected slowly into the substantia nigra (one injection per rat) and its effects on these responses were

observed for periods of 30 min–3 hr. In most cases the striatum was stimulated continuously during this time and histograms were computed at regular intervals.

'Chronic' experiments. In these studies the toxin was injected into the substantia nigra unilaterally and the effects of ionophoretically administered substances and striatal stimulation were subsequently determined on nigral neurones 2–10 hr after the injection. In many experiments control observations were made at corresponding times from the uninjected, i.e. contralateral, substantia nigra.

Effects of tetanus toxin on striatal neurones

The effects of tetanus toxin were also determined on ionophoretic drug responses and substantia nigra and dorsal raphe nucleus-evoked inhibition of striatal neurone firing following 'acute' and 'chronic' toxin administration. With the exception of the 'chronic' toxin experiments, the experimental protocol adopted was similar to that described above for the substantia nigra. The micropipettes used for the injection of tetanus toxin and recording neuronal activity in the substantia nigra experiments were often used subsequently in these experiments on the striatum.

'Chronic' experiments. Since the striatum is a large structure compared with the substantia nigra (eg. see Konig & Klippel, 1963) tetanus toxin was injected at three to four separate sites within the striatum. Recordings of neuronal activity were made 2–10 hr after these injections. Control observations were made from neurones in the contralateral striatum.

RESULTS

Effects of tetanus toxin on substantia nigra neurones

Controls. The effects of electrical stimulation of the striatum and ionophoretic administration of various putative neurotransmitter substances on substantia nigra neurones prior to the injection of toxin were similar to those previously reported in the rat (Crossman *et al.* 1973; Dray *et al.* 1976). Thus, in tests on seventeen neurones, spontaneous activity was consistently and reproducibly reduced by stimulation of the ipsilateral striatum. This inhibitory effect of striatal stimulation on substantia nigra neurones could also be produced when cells were excited by DLH administered ionophoretically indicating the post-synaptic nature of the inhibition. When the stimulus intensity was set at 1·5–2 times threshold the mean latency to onset of the inhibition was $6{\cdot}7 \pm 0{\cdot}5$ msec and the mean duration of the inhibitory period was $25 \pm 5{\cdot}2$ msec. In five neurones, synaptic inhibition was preceded by a brief period of excitation (mean duration 15 msec which occurred with a similar latency (mean $6{\cdot}0 \pm 1{\cdot}1$ msec) to the evoked inhibition described above). The duration of the inhibition which was preceded by an initial excitation was similar to that of inhibition alone. Dray *et al.* (1976) have discussed the possibility that this excitatory/inhibitory sequence observed in some neurones in the substantia nigra may be due to the simultaneous activation of an excitatory and an inhibitory striato-nigral pathway. The spontaneous activity of the seventeen neurones tested was also depressed by ionophoretic administration of GABA (5–30 nA) and glycine (10–50 nA), and activity was enhanced by DLH (2–25 nA). Ionophoretically administered 5-HT (45–100 nA) and dopamine (40–100 nA) depressed firing in four substantia nigra neurones. Neuronal responses to ionophoretically administered substances were considered to be genuine since they were not mimicked by the passage of positive or negative current through the dye-containing barrel of the micro-electrodes. In agreement with other reports which have indicated a neurotransmitter role for GABA in the striatonigral pathway (Precht & Yoshida,

1971; Crossman *et al.* 1973; Dray *et al.* 1976) bicuculline metachloride (15–30 nA) reversibly reduced both striatal-evoked inhibition and GABA-induced depression of spontaneous activity in five neurones. In contrast, strychnine (10–30 nA) only antagonized the depression of firing produced by glycine on the same neurones.

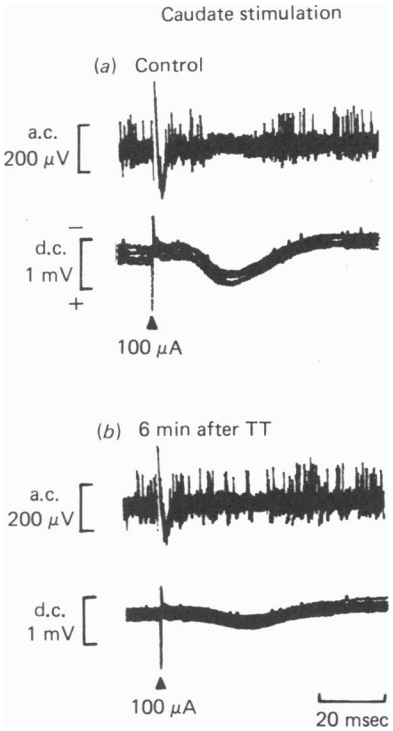

Fig. 1. Effects of an intranigral injection of tetanus toxin (10^2 mouse LD_{50}) on striatal-evoked inhibition of a substantia nigra neurone. In both *A* and *B* the upper trace shows the spike potential record (retouched) and the lower trace the accompanying d.c. field potential. (Each oscillographic record is composed of ten superimposed sweeps). *A*, inhibition of firing following stimulation of the ipsilateral striatum prior to the injection of tetanus toxin. Note the inhibitory pause in the a.c. record and the concomitant positive field potential on the d.c. record. *B*, the almost complete abolition of synaptic inhibition 6 min after the injection of tetanus toxin. The arrow below each record marks the position of the stimulus artifact. Tetanus was injected from a single micropipette located 700 μm from the recording micro-electrode. Note, the spike heights appear to vary in this record as they are superimposed on an oscillating base line.

'Acute' toxin effects. Following an intranigral injection of 10^2–10^3 mouse LD_{50} doses of tetanus toxin, striatal-evoked inhibition was abolished in fifteen of the seventeen neurones from which control observations had been made. An example of this effect of tetanus toxin on one neurone is shown in Fig. 1. No results were obtained from the remaining two neurones due to displacement of the recording electrode during the toxin injection. However, where tests were made, the sensitivity of the same neurones to ionophoretically administered GABA (eight cells), glycine (four cells), dopamine (four cells), 5-HT (four cells) and DLH (eight cells) was unaffected by the toxin (e.g. Fig. 2*F*). Tetanus toxin also failed to influence either

striatal-evoked excitation which preceded inhibition in five neurones or the level of background firing of all the neurones studied. In twelve experiments, the onset of action of the toxin was very rapid, synaptic inhibition being diminished within

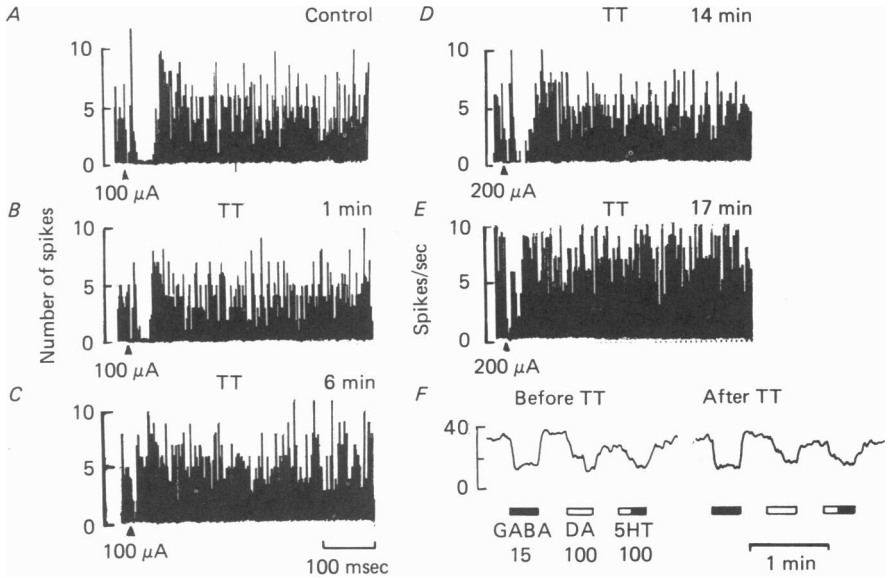

Fig. 2. The effects of tetanus toxin (TT) on the inhibition of the same substantia nigra neurone produced by stimulation of the ipsilateral striatum and ionophoresis of depressant substances. *A–D* are peristimulus histograms computed from fifty stimuli and analysed in 1 msec intervals. *E* is computed from eighty stimuli and analysed in 1 msec intervals. The position of the stimulus artifact is indicated by the arrow below each histogram. The break in the histogram in *A* corresponds to the striatal-evoked inhibition generated in this neurone during the control period before the injection of tetanus toxin. Histograms *B*, *C*, *D* and *E* were computed 1, 6, 14 and 17 min respectively after the intranigral injection of tetanus toxin (10^3 mouse LD_{50}). Note the absence of inhibition in *C* and the temporary reappearance of inhibition in *D* on doubling the striatal stimulating current. *F* is a ratemeter record of firing of the same nigral neurone and illustrates the depression of firing produced by ionophoresis of GABA (15 nA), dopamine (100 nA) and 5-HT (100 nA) before and 18 min after the injection of tetanus toxin. The length of the bars below this record indicates the duration of the ionophoretic ejections. Note tetanus toxin was injected from a micropipette cemented alongside a multibarrel micro-electrode (tip separation 500 μm).

1–4 min of the toxin injection and completely abolished 4–7 min later (Fig. 2*A–C*). In these experiments the striatum was stimulated continuously at 2 Hz until antagonism of the synaptic response was evident. In contrast, in three additional experiments when the striatum was stimulated intermittently, i.e. for 1 min at 2 Hz every 6 min, the onset of action of the toxin was delayed for 20, 25 and 45 mins respectively after the toxin injection. However, again complete antagonism of the evoked inhibition was apparent a few minutes later.

There was no recovery of the synaptic response in six neurones even though recordings were pursued for 2–3 hr after the effects of the toxin were first apparent. However, in six other neurones a temporary reversal of the toxin effect was observed when the intensity of the striatal stimulus was increased. This re-evoked synaptic

response was considerably attenuated (Fig. 2*D*) and declined with time (compare Fig. 2*E* with 2*D*).

In three experiments, once the effect of the toxin was apparent on the neurone under study, a search was made for further neurones. Six neurones encountered within 0·8 mm of the estimated injection site were inhibited by striatal stimulation

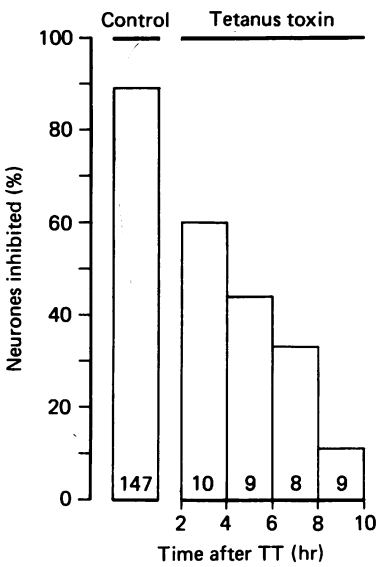

Fig. 3. The proportion of substantia nigra neurones inhibited by striatal stimulation at various times after the 'chronic' intranigral injection of tetanus toxin (TT; 10^3 mouse LD_{50} doses). The number in each column refers to the total number of neurones studied during each epoch and the height of each column represents the percentage of neurones inhibited. The column marked control contains the pooled results obtained from neurones in the contralateral uninjected substantia nigra. Further description of this Figure is given in the text.

of 1·5–2 times threshold. In each case, this evoked inhibition was gradually reduced within 6–20 min of first locating the cell. This effect was not accompanied by any change in sensitivity to ionophoretically ejected substances.

Effects of neutralized tetanus toxin. To determine whether the effects of the toxin outlined above could be accounted for by some contaminant in the toxin solution or were due to failure of prolonged striatal stimulation to evoke inhibition, tetanus toxin previously neutralized with antitoxin was injected unilaterally into the substantia nigra of three rats. The neutralized toxin had no demonstrable effect on synaptic inhibition evoked in six neurones in these animals although recordings were pursued for 1–3 hr after the injection.

'Chronic' toxin effects. The effects of 'chronic' intranigral toxin administration on striatal-evoked synaptic inhibition are summarized in Fig. 3. Of nineteen neurones recorded 2–6 hr after the toxin injection, ten were inhibited by striatal stimulation, whereas 6–10 hr after the toxin injection only three of seventeen neurones encountered were inhibited. The three neurones in the latter case were inhibited by stimulus intensities that were not significantly different from those found for the control contralateral nigra. Unresponsive neurones were unaffected by striatal stimuli of

400–600 μA intensity, i.e. supramaximal stimuli. In contrast to these findings, twenty-seven of thirty neurones encountered in the control contralateral substantia nigra of these rats were inhibited by striatal stimuli of 20–100 μA. For convenience, these control results are all included in the first bar of the histogram in Fig. 3.

The sensitivities of neurones to ionophoretically administered GABA (10–25 nA), glycine (10–30 nA), dopamine (70–100 nA) and 5-HT (70–100 nA) were similar in

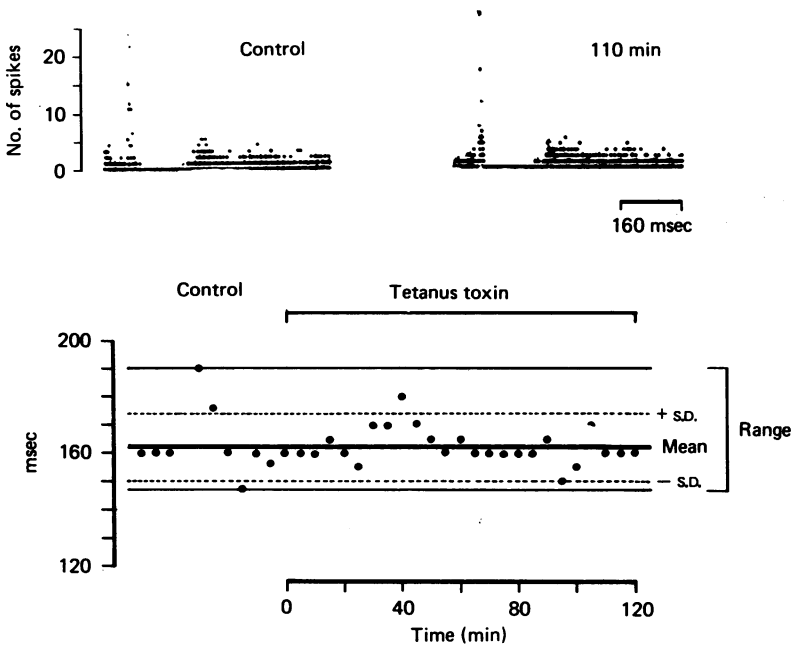

Fig. 4. The effects of an intrastriatal injection of tetanus toxin (10^3 mouse LD_{50}) on nigral-evoked responses of a striatal neurone. The lower record is a graphical representation of the evoked inhibition before and after tetanus toxin injection. The inhibitory periods are shown on the ordinate and time scales on the abscissa. Each dot on the graph represents the duration of the inhibitory period measured from a peri-stimulus histogram constructed at different times. The mean standard deviation (S.D.) and range of ten inhibitions during the control period before the toxin injection are presented on the graph as continuous or interrupted horizontal lines. During a period of 120 min after the injection of toxin the inhibitory periods were not significantly different from the control values. The upper records are histograms of the responses of the same neurone to nigral stimulation before (control) and 110 min after toxin injection. Note the preceding excitation (peak) and evoked inhibition (trough) are unaffected by the toxin. Both histograms were computed from 100 sweeps analysed in 0·6 msec intervals.

both toxin-treated and control substantia nigra. Furthermore, the mean spontaneous background firing rate of neurones in the toxin-treated substantia nigra (41·3 $\pm$ 3·8 S.E. spikes/sec) was not significantly different from that in the control nigra (37·3 $\pm$ 2·9 spikes/sec).

Effects of tetanus toxin on striatal neurones

The majority of striatal neurones studied in this part of the investigation were driven continuously by ionophoretic DLH (0–6 nA) to give stable base line firing

rates of about 10 Hz. As reported in an earlier study (Davies & Tongroach, 1978) stimulation of the substantia nigra evoked an early excitation followed by inhibition of firing while dorsal raphe nucleus stimulation evoked only inhibition.

'Acute' toxin effects. The effects of a unilateral injection of tetanus toxin (10^3 mouse LD_{50}) into the striatum was observed on a total of fifteen striatal neurones which exhibited excitatory/inhibitory responses following substantia nigra stimulation. In each individual neurone some variations occurred in the evoked inhibitory period. However, peristimulus histograms regularly computed up to 2·5 hr after the toxin injection revealed no significant change in either the latency to onset or the duration of the evoked inhibition or preceding excitation in any of these neurones compared to the corresponding control pre-toxin values. The results obtained on one striatal neurone are illustrated in Fig. 4.

Dorsal raphe nucleus stimulation evoked inhibition in eight of the fifteen neurones mentioned above. This inhibition was also unaffected by tetanus toxin. Furthermore, tetanus toxin did not appear to modify the depressant effects of ionophoretic dopamine, 5-HT or GABA in tests on five of these neurones.

'Chronic' toxin effects. In experiments on four animals the effects of ipsilateral substantia nigra stimulation and dorsal raphe nucleus stimulation were investigated on striatal neurones 3–10 hr after the injection of 1 μl. tetanus toxin (10^3 mouse LD_{50} per μl.) at three or four different sites in the striatum. In thirteen of fifteen neurones studied, nigral stimulation evoked sequences of excitation followed by inhibition, the characteristics of which were similar to those evoked in the control contralateral striatum (ten cells studied). In the remaining two neurones, nigral stimulation evoked a prolonged period (120–240 msec) of excitation occurring after a latency of 8 and 10 msec respectively. In tests on eleven neurones dorsal raphe nucleus stimulation evoked clear inhibition in seven, which was similar in character to that evoked in four of ten neurones studied in the control contralateral striatum. The remaining four cells in the toxin-treated striatum and six cells in the control striatum were unaffected by dorsal raphe nucleus stimulation.

The effects of ionophoretically administered dopamine, 5-HT and GABA were tested on five neurones. These three putative transmitters consistently and reproducibly depressed the firing of all five neurones. The onset times of the drug effects and ionophoretic currents required to produce 80–100% depression of DLH-induced activity were similar to those observed on eight neurones in the control contralateral striatum.

DISCUSSION

The present results show that tetanus toxin abolishes bicuculline-sensitive, GABA-mediated, striatal-evoked inhibition of substantia nigra neurones. Control observations made with tetanus toxin neutralized with antitoxin indicate that tetanus toxin itself, and not some contaminant, blocks synaptic transmission in the substantia nigra. These findings are similar to those reported with tetanus toxin on GABA-mediated inhibition of cerebellar Purkinje cells (Curtis *et al.* 1973) and, in contrast to earlier studies in the cerebral cortex (Krnjević *et al.* 1966) and Deiters' nucleus (Obata *et al.* 1967), indicate that tetanus toxin abolishes GABA-mediated synaptic inhibition at supraspinal sites.

The inability of tetanus toxin to influence synaptic inhibition produced by surface stimulation in the cerebral cortex (Krnjević *et al.* 1966) may be explained if the pathway involved is not GABA-ergic. The observation that the GABA antagonist, picrotoxin, failed to block the inhibitory effect of surface stimulation on cortical neurones (Krnjević *et al.* 1966) is consistent with this suggestion. Furthermore, the report that bicuculline interferes with this cortical inhibition (Curtis & Felix, 1971) is not necessarily at variance with this proposal since it would appear that the two groups of investigators were studying the inhibitory effects of surface stimulation on different cortical neurones. Curtis & Felix (1971) limited their study to identified pyramidal tract neurones whereas Krnjević *et al.* (1966) examined the effects of surface stimulation on unidentified cortical neurones. Regarding the inhibitory pathway from cerebellar Purkinje neurones to Deiters' nucleus, available evidence strongly favours GABA as the transmitter substance involved (Curtis & Johnston, 1974). It is, therefore, difficult to reconcile the absence of effects of tetanus toxin on this inhibitory pathway (Obata *et al.* 1967) with the present findings in the substantia nigra. However, it may be significant that the effects of locally administered tetanus toxin were only examined in one experiment on Deiters' nucleus.

Previous studies indicate a presynaptic site of action for tetanus toxin (Curtis & De Groat, 1968; Curtis *et al.* 1973; Mellanby & Thompson, 1972). The present observations that an injection of the toxin into the substantia nigra abolishes synaptic inhibition without affecting the post-synaptic sensitivity of nigral neurones to GABA and other putative neurotransmitters is consistent with this suggestion.

The present 'chronic' series of experiments indicates that complete antagonism of striatal-evoked inhibition in the substantia nigra occurs 4 hr or more after an intranigral injection of toxin. This finding is in accord with other studies involving local toxin injections into different areas of the central nervous system (Brooks & Asanuma, 1965; Curtis *et al.* 1973). Such factors as the rate of spread of the toxin in nervous tissue probably contributes significantly to the time of onset of action of the toxin. However, the rapid 4–7 min onset action of the toxin observed in the 'acute' experiments on the substantia nigra is considerably shorter than the 50–60 min onset times reported in earlier studies on the cerebellum and spinal cord (Curtis & De Groat, 1968; Curtis *et al.* 1973). Differences in the doses of toxin administered, the brain area studied or the species used may account for these discrepancies, although these are not very satisfactory explanations. The discrepancies could be due to the stimulus intensities employed to evoke inhibition. In this respect it was observed in this study that increasing the stimulus strength often resulted in the reappearance of inhibition, suggesting that the time of onset of the toxin action would have appeared longer had higher stimulus intensities been used initially. Another factor which may have contributed to the differences in the time of onset of the toxin action was the rate of activation of the synaptic pathway. It was observed in three experiments that the rate of onset of action of the toxin was considerably longer (20–45 min) when the striato-nigral pathway was activated intermittently rather than continuously, which is compatible with this suggestion. Other studies indicate that tetanus toxin is bound to presynaptic nerve terminals (Price, Griffin & Peck, 1977) and that the rate of ascent of the toxin through axons is increased by neuronal activity (Wellhoner, Seib & Hensell, 1973). It is possible,

therefore, that the rate-limiting step regarding the time of onset of the toxin action is dependent upon the rate of uptake and binding of the toxin to presynaptic terminals, which is determined by the frequency of activation of the inhibitory pathway. Interestingly, the rate of uptake of another protein, horseradish peroxidase, into axon terminals depends upon the frequency of activation of the axon (Dolivo, Meurant & Verdan, 1977).

The possible presynaptic mechanism of action of tetanus toxin has been discussed at length by Curtis (1971) in relation to the abolition of spinal inhibition which is sensitive to strychnine. He concludes that the toxin may interfere with the synthesis or release, or both, of the inhibitory neutrotransmitter, glycine. Similar mechanisms could account for the action of the toxin on the GABA-mediated inhibition in the substantia nigra. In the spinal cord the levels of GABA are unaffected following the development of local tetanus (Johnston, De Groat & Curtis, 1969; Semba & Kano, 1969), suggesting that interference with synthesis is unlikely to account for the actions of tetanus toxin. In keeping with this, we were unable to detect any change in the levels of GABA in the substantia nigra 6 hr after an intranigral injection of tetanus toxin (G. G. S. Collins, J. Davies & P. Tongroach, unpublished results). On the other hand, the electrically stimulated release of GABA and glycine from synaptosomes isolated from the medulla and spinal cord of rats treated with tetanus toxin is significantly reduced (Osborne, Bradford & Jones, 1973) and potassium-evoked release of GABA from nigral slices of rats pre-treated with tetanus toxin is also reduced (J. Davies, P. Tongroach & M. J. Neal, unpublished results). Thus it would seem most probable that the toxin interferes with transmitter release although the mechanism(s) responsible for such an effect remains to be determined. Interference with terminal excitability is unlikely to be responsible for such an effort, as evoked excitation which sometimes preceded striatal-evoked inhibition was not influenced by the toxin. However, the possibility that tetanus toxin selectively affects terminal excitability of GABA-ergic terminals cannot be ruled out.

Previous studies have demonstrated that tetanus toxin is without effect on excitatory synaptic events in spinal neurones (Brookes, Curtis & Eccles, 1957; Wilson, Diecke & Talbot, 1960; Curtis & De Groat, 1968; Curtis, Game, Lodge & McCulloch, 1976). The lack of effect of the toxin on stimulus-evoked excitation in the striatum and substantia nigra in the present experiments suggests that synaptic excitation at supraspinal sites is also uninfluenced by the toxin. Earlier investigations have indicated that inhibition of striatal neurones evoked by stimulation of the substantia nigra and dorsal raphe nucleus is mediated by dopamine and 5-HT respectively (Bloom *et al.* 1965; McLennan & York, 1967; Connor, 1970; Feltz & de Champlain, 1972; Gonzales-Vegas, 1974; Liles, 1974; Miller *et al.* 1975; Zarzecki *et al.* 1976; Olpe & Koella, 1977; Davies & Tongroach, 1978). The present 'acute' experiments on the striatum demonstrate that tetanus toxin does not affect these monoamine-mediated inhibitions when administered in doses, and often from the same micropipettes, that abolished synaptic inhibition in the substantia nigra. This observation suggests that tetanus toxin has a selective action on amino-acid-mediated inhibitory synaptic events. Failure to evoke inhibition in about 40% of striatal neurones by dorsal raphe nucleus stimulation in 'chronic' experiments is not at variance with this suggestion since raphe stimulation only evoked inhibition

in 40% of neurones in the control striatum. On the other hand, the absence of nigral-evoked inhibition in two striatal neurones in these 'chronic' experiments may have been the result of an action of the toxin. This is not necessarily due to an interference with dopaminergic neurotransmission however, as there is some evidence for a bicuculline-sensitive nigral-evoked inhibition in the striatum (Feltz, De Champlain & Dessama, 1975). Nevertheless, further investigations on other chemically identified synaptic inhibitions are necessary to establish the specificity of the toxin as an antagonist of amino-acid-mediated synaptic inhibitions. Interestingly, peripheral studies indicate that noradrenergic transmission is unaffected by the toxin (Ambache, Morgan & Wright, 1948; Mellanby, Pope & Ambache, 1967); however, whether this is pertinent to the central nervous system remains to be determined.

Tetanus toxin has been used as a histochemical tool for tracing fibre connexions in the central nervous system (Schwab, Agid, Glowinski & Thoenen, 1977) and has also recently been used as a marker for neurones in culture (Mirsky, Wendon, Black, Stolkin & Bray, 1978). The present finding indicates that it may be a very useful and selective tool for the identification of amino-acid-mediated synaptic inhibition. Furthermore, it may be possible to lesion pathways utilizing inhibitory amino acids as neutrotransmitters with the toxin in a way somewhat analogous to the use of 6-hydroxydopamine for catecholamine pathways.

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