Antagonism of synaptic inhibition in the rat substantia nigra by tetanus toxin

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It is known that tetanus toxin blocks strychnine-sensitive postsynaptic inhibition in the spinal cord (Brooks, Curtis & Eccles, 1957). This action of the toxin is believed to be due to a reduced release of the inhibitory transmitter glycine (Curtis & De Groat, 1968). Recently, it has been suggested that the toxin also reduces the release of GABA since it blocks bicuculline-sensitive inhibition in the spinal cord and cerebellum (Curtis, Felix, Game & McCulloch, 1973). In the present experiments the effects of tetanus toxin were investigated on caudate evoked post-synaptic inhibition in the substantia nigra where there is good evidence that GABA is the inhibitory transmitter (see Dray, Gonye & Oakley, 1976).

Experiments were performed on rats anaesthetized with urethane (1.2–1.4 g/kg, i.p.). Extracellular recordings were made from single nigral neurones from the centre barrel (4 M NaCl) of a 7 barrel microelectrode. The following substances were ejected from the outer barrels using standard microelectrophoretic techniques: Acetylcholine Cl (1 M), GABA (0.5 M pH 3.5), dopamine HCl (DA 0.5 M), 5-hydroxytryptamine bimaleate (5-HT 0.5 M) bicuculline methochloride (BMC 0.005 M in 0.165 M NaCl), DL-homocysteate (DLH 0.2 M pH 7), tetanus toxin (1.5×10^2 mouse MLD in 0.165 M NaCl). Tetanus toxin (Burroughs Wellcome) was administered either via a micrometer syringe and a glass pipette (tip dia. 20–30 μ) attached to the multibarrel electrode such that the latter projected 500–800 μM beyond the toxin pipette or by microelectrophoresis from one barrel of the microelectrode. Postsynaptic inhibition was evoked on substantia nigra neurones by single 100 μA pulses ($2\ s^{-1}$, 100–300 μs) via a bipolar stimulating electrode positioned in the ipsilateral caudate nucleus.

Single microinjections (0.5–1 μl) of 10^2–10^3 mouse MLD of toxin abolished synaptic inhibition evoked in 10 nigral neurones within 4–7 minutes. The toxin had no discernible effect on spontaneous firing rates or on responses induced by ejections of DLH, GABA, DA or 5-HT. By contrast, BMC (20–50 nA for 2–25 min) caused 50–100% antagonism of synaptic inhibition on 7 neurones and simultaneously abolished responses to electrophoretically ejected GABA. Administered electrophoretically tetanus toxin was much less effective in antagonizing synaptic inhibition than when administered by microinjection. Hence, 100–200 nA toxin ejected for 45–60 min only partially reduced inhibition in 3 neurones and was without effect on 2 neurones.

These results provide further evidence that tetanus toxin antagonizes GABA mediated inhibition in the central nervous system by a presynaptic action.

This work was supported by the Medical Research Council.

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Use of protease inhibitors to protect subcutaneously injected peptide hormones against local degradation

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Although peptide hormones are usually administered subcutaneously, the extent to which they are degraded before absorption has been insufficiently studied. Local monitoring of the injection site after giving isotopically labelled hormone indicates that radioactivity disappears rapidly (Binder, 1969), but it cannot be assumed that the labelling atoms remain within bioactive molecules. Data obtained by radioimmunoassay also requires critical evaluation because of the lack of correlation between immunological and biological activity in many peptide fragments, and few bioassays are sufficiently sensitive to follow blood levels.

We have studied the effect of protease inhibitors on local degradation, using bovine parathyroid hormone (bPTH 1–84) and synthetic amino-terminal fragments of the bovine and human sequences (bPTH 1–34 and