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.

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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

hPTH 1–34) (Tregear, Reitschoten, Greene, Keutmann, Niall, Reit, Parsons & Potts, 1973; Niall, Sauer, Jacobs, Keutmann, Segre, O'Riordan, Aurbach & Potts, 1974) in the subcutaneous version of a bioassay depending on measurements of hypercalcaemia in chicks (Parsons, Reit & Robinson, 1973). Both Trasylol and ϵ-amino caproic acid (EACA) substantially enhanced the hypercalcaemia measured 2 h after injection. It was confirmed by radioimmunoassay that this enhancement reflected an increase in circulating blood levels of hPTH 1–34, the greatest increase being seen 1 h after injection. Similar enhancement in circulating blood levels of another peptide hormone was observed when EACA and Trasylol were added to porcine calcitonin, injected subcutaneously to chicks. Large doses of calcitonin can be given to these birds and the resulting blood levels were followed directly by injecting their plasma intravenously to rats and measuring the hypocalcaemic response as described by Kumar, Slack, Edwards, Soliman, Baghdiantz, Foster and MacIntyre (1965).

A quite independent approach to the evaluation of subcutaneous losses appears to be possible by using controlled intravenous infusion to imitate the changing blood levels measured after s.c. injection. The intravenous technique allows certainty that all hormone administered enters the bloodstream and comparisons of s.c. and i.v. administration in freely mobile dogs with indwelling venous cannulae have yielded preliminary evidence to confirm that substantial local destruction must occur when hPTH 1–34 is injected subcutaneously.

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Drug clearance in the rabbit twenty-four hours after an intoxicating dose of ethanol

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Acute ethanol intoxication is known to inhibit the *in vivo* metabolism of a variety of drugs, including barbiturates, in animals and in man (Rubin, Gang, Misrad & Lieber, 1970), although it is not so clear whether this is a direct inhibitory effect of the ethanol itself. The levels of ethanol required to inhibit the metabolism of most drugs by the hepatic microsomal fraction, around 100 mM (Rubin *et al.,* 1970; Cinti, Grundin & Orrenius, 1973), are in general higher than those encountered except under cases of severe intoxication. It was of interest therefore to examine *in vivo* drug clearances several hours after a single dose of ethanol, when the ethanol had been completely removed from the blood.

Rabbits were fed ethanol (4.25 g/kg) as a 50% solution (v/v) by stomach tube. A peak blood ethanol concentration (mean $\pm$ s.e. mean, $n = 3$) of 397 ± 7 mg/100 ml was achieved within 2 h and had fallen to 1.3 ± 0.9 mg/100 ml by 24 hours. Blood acetaldehyde levels after 24 h, 0.11 ± 0.01 mg/100 ml, were no different from control values 0.09 ± 0.01 mg/100 ml ($P > 0.05$). Hexobarbitone sodium (50 mg/kg) administered intravenously, was removed from the blood with a half life (mean $\pm$ s.e. mean, $n = 7$) of 22.9 ± 1.3 min in control animals and 41.0 ± 5.8 min ($n = 4$, $P < 0.01$) in animals pretreated with ethanol 24 h previously. There appeared to be no significant difference in the half life for the removal of intravenously administered aniline hydrochloride (50 mg/kg) between control 29.5 ± 1.2 min ($n = 6$) and ethanol pretreated animals 34.1 ± 2.6 min ($n = 4$, $P > 0.05$). The decrease in the rate of removal of hexobarbitone could not be accounted for by any change in the hepatic microsomal metabolism of hexobarbitone measured *in vitro,* control 2.3 ± 0.1, ethanol pretreated 2.2 ± 0.1 nmol min^{-1} mg protein^{-1} ($n = 7$, $P > 0.05$) or in the levels of microsomal cytochrome P-450, control 1.0 ± 0.1 ethanol pretreated 1.0 ± 0.1 nmol mg protein^{-1} ($n = 3$). Microsomal aniline hydroxylation was similarly unchanged,