Central actions of valproate sodium

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Valproate sodium (VPA) has proved to be an efficient antiepileptic drug especially active against generalized seizures of the absence-type (Simon & Penry 1975). Biochemical results suggest that its mechanism of action involves GABA. Thus Löscher & Frey (1977) noted that VPA induces an elevation of the brain GABA-concentration and that it increases activity of glutamic acid decarboxylase, whereas GABA-transaminase was found to be inhibited, but only when toxic doses of VPA were used. Anlezark et al (1976) are of the opinion that the VPA-induced increase in brain GABA-concentration may be due to a competitive inhibition of brain succinic semialdehyde dehydrogenase. In Harvey's view (1976), the enhancement in brain GABA-concentrations after VPA could be the result of reuptake-inhibiting properties of this drug.

In view of these suggested "GABA" enhancing effects of VPA—notwithstanding the intimate biochemical mechanisms involved—we have investigated with a neurophysiological-pharmacological methodology, the influence of VPA on central effects of GABA in rats. In particular, using microiontophoretic techniques, we studied the modulation of GABA effects by (systemic or local) application of VPA on single nerve cells. For comparison the influence of VPA on glycine-induced effects was also examined. Finally the time course of the effect of systemically applied VPA on single units was compared with that exerted on electroshock-induced convulsions.

The anticonvulsive property of VPA was determined in groups of 20 rats. The drug was administered intraperitoneally (i.p.) at a dose of 200 mg kg^{-1}. The number of protected animals for each time (1, 3, 5, 10 and 15 min before supramaximal electroshock-treatment) was checked. The action of VPA at a dose of 200 mg kg^{-1} i.p. on the spontaneous discharge-rate of unidentified neocortical neurons was investigated in 6 rats anaesthetized with chloral hydrate, 400 mg kg^{-1} i.p. The mean change of the firing-rate of neocortical cells and the anticonvulsive effect of VPA as a function of time are shown in Fig. 1. Note that the two time-courses are similar.

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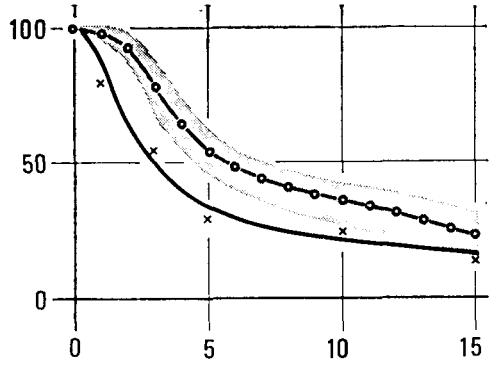

FIG. 1. Upper curve: Time course of the inhibitory effect of VPA (200 mg kg^{-1} i.p.) on the spontaneous firing-rate of single neocortical neurons in rats. ○, n = 6 ± s.e.m. Ordinate: percentage spontaneous cell-activity. Abscissa: time (min).

Lower curve: Time course of the anticonvulsive effect of VPA (200 mg kg^{-1} i.p.) on electroshock-induced convulsions in rats. X, n = 20/t unit. Ordinate: percentage convulsing rats. Abscissa: time (min).

For iontophoresis, drugs were applied through 3–4 barrelled micropipets on unidentified neocortical cells. Ejection currents were balanced during all drug administrations. The following aqueous solutions were used: GABA (0·5 M, pH 3·5); glycine (0·5 M, pH 3·5); VPA (0·5 M, pH 8·0). Iontophoresed VPA per se had either no or only a slight excitatory influence on the firing-rate of the neurons. However, the inhibitory effect of microiontophoretically applied GABA on neocortical cells was significantly enhanced and prolonged by simultaneously iontophoresed VPA (20 cells; enhancement of the firing-depression: mean 18·6%, $P < 0.001$ vs control; prolongation of duration of GABA-inhibition: mean 49·3%, $P < 0.001$ vs control). On the other hand, the inhibitory effect of a submaximal "dose" of iontophoresed GABA on neocortical cells was not potentiated by VPA at a dose of 100 mg kg^{-1} i.p. (6 rats). The inhibitory action of glycine was unaffected by locally applied VPA (11 cells).

The effect of VPA on transsynaptic GABAergic transmission was investigated on neurons in substantia nigra receiving a GABAergic striatal input and on neurons of the Deiter's nucleus being inhibited by afferents originating from Purkinje cells. Post-stimulus-time histograms were repeatedly recorded from each neuron over a period of at least 15 min. Transsynaptic inhibition of nigral cells induced by electrical stimulation of the striato-nigral GABAergic fibre tract was potentiated in only 4 out of 15 cells by microiontophoresed VPA. Intraperitoneally given VPA (200 mg kg^{-1}) potentiated slightly this inhibition in only 1 out of 8 cells. Transsynaptic GABAergic inhibition of cells in Deiter's nucleus induced by weak electrical stimulation of the cerebellar cortex was not influenced at all by i.p. VPA (200 mg kg^{-1}; 5 neurons). The GABAergic nature of this transsynaptic inhibition was proven by application of bicuculline.

In the present study the inhibitory action of the iontophoretically applied neurotransmitter GABA was modulated by iontophoresed VPA. If reliance is placed on the effect observed in these experiments it may

inferred that VPA indeed acts via potentiation of GABAergic transmission. However, this interpretation cannot be sustained on the basis of the experiments dealing with the interaction of intraperitoneally given VPA with iontophoresed GABA and of VPA with GABA-mediated transsynaptic inhibition. Assuming that VPA acts through potentiation of GABAergic transmission one would expect a potentiation of GABAergic transsynaptic inhibition when the drug is applied in anticonvulsant doses. However, in our experiments we did not observe such an effect. Thus, in conclusion, our results indicate that the anticonvulsive mode of action of VPA is not mainly due to a potentiation of central inhibitory GABAergic transmission. Alternatively, a direct effect of VPA upon neuronal membrane properties has been suggested recently (Slater & Johnston 1977).

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Transport of liposome components in rat everted intestinal loops

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Many therapeutic agents that are poorly absorbed or biodegraded in the intestine have to be given parenterally with inconvenience and risk. A novel approach has recently been indicated through the use of phospholipid vesicles (liposomes) (Tyrrell et al 1976; Patel & Ryman 1976; Gregoriadis 1976; Dapergolas & Gregoriadis 1976; Dapergolas et al 1976). Oral administration of liposome-entrapped insulin caused a significant reduction of blood sugar concentrations in diabetic rats. However, in normal rats the insulin entrapped in egg yolk lecithin vesicles failed to produce such an effect (Patel & Ryman 1976; Dapergolas & Gregoriadis 1976). A possible explanation for the differences in the results between normal and diabetic rats could have been due to a difference in the rate of liposome transport through the gut wall. To test this possibility, we studied the transport of radioactively labelled constituents of liposomes through rat everted intestinal loops.

The efficiency of insulin entrapped in liposomes in lowering blood glucose concentration depended on the composition of the liposomes (Dapergolas & Gregoriadis 1976; Dapergolas et al 1976; Gregoriadis 1976). This also might be due to differences in the rate of uptake of various liposomes preparations through the gut wall. To examine this possibility, we tested the permeability of everted intestinal loops to labelled cholesterol and phosphatidylcholine incorporated in two types of liposomes, namely large multilamellar vesicles (LMV) and small unilamellar vesicles (SUV) and composed of various phospholipids.

Pure egg phosphatidylcholine (Egg PC, Makor Chemicales, Jerusalem), dipalmitoyl phosphatidylcholine (DPPC, Sigma), dicetylphosphate (DCP, Sigma) and cholesterol (CH, Merck), were obtained as commercial products and were used without further purification. Labelled ^{14}C-CH (CH, 57·7 mCi mol^{-1}) was purchased from Amersham and ^{14}C-PC (PC, 50–60 mCi mol^{-1}) was prepared according to Asher et al (1969).

Liposomes were prepared from PC and CH in the absence or presence of DCP (molar ratio—7 : 2 : 1). They were labelled either by ^{14}C-CH or by ^{14}C-PC. The components were dissolved together in chloroform, the solutions evaporated to dryness under vacuum and the residue dispersed in Krebs solutions to a final PC concentration of 5% (w/v), unless otherwise specified. Multilamellar liposomes (LMV) were obtained by mixing the aqueous suspensions. Small unilamellar vesicles (SUV) were prepared by sonication of the latter aqueous dispersions (by Heat System Model 350 sonicator) until they were clear (10–20 min).

Everted intestinal loops were prepared from normal and diabetic albino rats (150–180 g) of the Hebrew University (Sabra) strain. Experimental diabetes was achieved by subcutaneous injection of either alloxan (180 mg kg^{-1}) or by intravenous injection of streptozocin (50 mg kg^{-1}) and the rats were killed 2 or 9 days following the injection, respectively. The intestine was removed, rinsed with saline and everted. Loops of 5 cm length each were tied at the ends, after being filled with 0·7 ml Krebs solution and 0·3 ml of CO_2 (5%) in oxygen.

The everted loops were immersed in 10 ml of liposome dispersions in Krebs solution in 25 ml flasks which had been bubbled before with 5% CO_2 in oxygen. The dispersions were incubated for 1 h in a shaking bath at 37 °C. From each loop, 0·5 ml was then taken by syringe and transferred to a vial containing 3 ml Insta-Gel (Packard) and counted in a β-liquid scintillation counter (Packard, Model 2003). 0·05 ml of the

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