Anti-epileptic properties of sodium valproate in rat amygdaloid kindling

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Kindling is a term which has been applied to the progressive induction of epileptiform seizures by repeated low intensity stimulation of discrete brain regions, most notably the amygdala (Goddard, McIntyre & Leech, 1969; Racine, 1972). In this study we present an electrographical and behavioural assessment of the effectiveness of the anti-convulsant, sodium valproate (Epilim) in this model of experimental epilepsy.

Bipolar electrodes were implanted chronically into the basolateral amygdala of male Sprague–Dawley rats (280–300 g); one group received daily stimulation for 2 s (biphasic d.c. pulses, 100 μA; 1 ms duration; 62.5 Hz—standard stimulation) until they displayed reproducible generalised clonic convulsions comprising forelimb clonus, rearing and loss of posture (mean number of days = 11). Amygdaloid EEG was recorded differentially between the electrode terminals. The effect of sodium valproate on this fully kindled epilepsy was investigated in dose groups of 8 rats. Control convulsions and after-discharges (ADs) were triggered on three successive days; on the fourth day one of the following—sodium valproate, 200, 400, 600 mg/kg or drug vehicle (dist, H_2O)—was administered orally and 60 min and 24 h later a further standard stimulation was given. Valproate, 400 and 600 mg/kg significantly decreased in a dose-related manner both the duration of AD ($P < 0.01$) and the incidence of forelimb clonus and full convulsions at 60 min but not at 24 h, post-treatment.

A further experiment examined the influence of valproate on epileptogenic threshold. AD threshold currents were determined in 6 kindled rats (mean current, 63 ± 8.2 μA peak to peak). Valproate (400 mg/kg, p.o., 1 h before stimulation) elevated the AD threshold by 10–20% in 5 animals.

To study the effect of valproate on kindling development, groups of 6 rats, previously unstimulated, were dosed orally once daily on 14 consecutive days with one of the following: sodium valproate, 100, 200, 400 and 600 mg/kg or dist. H_2O, 1 h before delivery of standard kindling stimulation. Following cessation of drug treatment rats were stimulated daily until they all showed full convulsions. Valproate (200, 400 and 600 mg/kg) significantly retarded AD development during the treatment period ($P < 0.01$) in a dose-dependent manner; and no convulsions occurred in the two top dose-groups. At both these doses there was a post-treatment dissociation of AD activity from convulsive behaviour: ADs attained full maturity within 3 days post-treatment, but the mean total number of days to full seizure was 30 in both groups, compared with 11 days in the control group.

These findings demonstrate that sodium valproate has an anti-epileptic action on the kindled epileptic focus which correlates well with the observed anticonvulsant effect. Valproate also has a pronounced prophylactic effect on kindled seizure development.

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PGE_2 mediated negative feedback of noradrenaline overflow in the central nervous system

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Evidence for a prostaglandin E_2 (PGE_2) mediated negative feedback control of noradrenaline (NA) release is plentiful in the peripheral nervous system (see Hedqvist, 1977). Less data are available for the central nervous system. Previously, using rat brain synaptosomes, no effect of PGE_2, $PGF_{2\alpha}$ or indomethacin on the spontaneous or K^+ evoked overflow of [^{3}H]-NA was evident (Hillier, Roberts & Templeton, 1979). This work has now been extended to study the effects of PGs, indomethacin, and the endoperoxide analogue U44619 ((15S)-hydroxy-11α, 9α-(epoxymethano) prosta-5Z, 13E-dienoic acid) on rat brain cortex slices (0.2 mm × 0.2 mm) preloaded with [^{3}H]-NA. Spontaneous efflux of [^{3}H]-NA from slices was similar to synaptosomes, but the Ca^{2+} dependent

increase in overflow evoked by 30 mM K^+ was 4–5 × the basal level in slices compared with only 1.5–2 × the basal level in synaptosomes ($n > 12$).

PGE_2 (2.8×10^{-5} M) and indomethacin (5.6×10^{-5} M) were without effect on the spontaneous efflux of [^{3}H]-NA from slices, however the 30 mM K^+ evoked overflow was increased by indomethacin (5.6×10^{-6} M) to 139.3 ± 10.7% (mean ± s.e.) of control levels ($P < 0.05$) while PGE_2 (2.8×10^{-6} M) reduced the overflow to 70.5 ± 1.6% of control ($P < 0.001$). PGE_2 (5.6×10^{-6} M) and U46619 (2.85×10^{-6} M) also reduced the evoked overflow, but not significantly. Since indomethacin increased the overflow, endogenous PGs may normally limit NA release and thus could mask the effects of exogenous PGs. The effect of PGE_2 was, therefore, studied in preparations where endogenous PG synthesis was blocked throughout by indomethacin (5.6×10^{-5} M). PGE_2 (0.28, 1.4, 2.8, 5.6, 14.0 and 28.0×10^{-6} M) reduced the evoked overflow to 83.7 ± 5.1 (n.s.), 78.6 ± 5.6 (n.s.), 57.9 ± 16.2 ($P < 0.05$), 56.3 ± 1.8 ($P < 0.01$), 55.8 ± 9.8 ($P < 0.05$) and 60.7 ± 13.6 ($P < 0.05$)% of control respectively ($8 \geqslant n \geqslant 4$).

The reduction in [^{3}H]-NA overflow by PGE_2 could be partially prevented by addition of the PG receptor blocker SC19220 (1-acetyl-2-(8-chloro-10,11-dihydrodibenz[b, f][1, 4]oxazepine-10-carbonyl) hydrazine) to the perfusion medium. Using indomethacin treated preparations, in the presence of SC19220, PGE_2 (0.28, 1.4, 2.8 and 14.0×10^{-6} M) now reduced overflow to 101.7 ± 22.2, 88.5 ± 17.7, 87.4 ± 5.5 and 87.9 ± 6.3% of control respectively ($6 \geqslant n \geqslant 5$). The effects of PGE_2 were now not significantly different from control at any concentration.

It is unclear why the PGE_2 mediated feedback should be evident in slices but not in synaptosomes. The discrepancy may perhaps be attributable to the difference in preparation times (0.25 versus 3.5 h) and the greater mechanical disruption required for the preparation of synaptosomes.

It appears that a PGE_2 mediated negative feedback, similar to that shown in peripheral systems, does operate in the central nervous system, however, the physiological importance of this effect has not been ascertained.

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Release of [^{3}H]-noradrenaline from the guinea-pig vas deferens by ethacrynic acid

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Ethacrynic acid (EA), a potent diuretic and Na^+, K^+-ATPase inhibitor, increases the force and rate of contractions of isolated atria (Pousti, Zarrindast, Sadeghi & Khoyi, 1973; Khoyi, Pousti, Powis & Zarrindast, 1978) and contracts the vas deferens of the guinea-pig (Khoyi, Pousti & Zarrindast, 1974). The cardiac effects are blocked by propranolol or reserpine but not by desipramine or colchicine. The effect on the vas deferens is prevented by phentolamine or reserpine. In the present work, the effect of EA on the release of tritium from guinea-pig vasa deferentia preloaded with [^{3}H]-noradrenaline was studied. EA (200 μg/ml) increased the rate of tritium outflow from 0.49 ± 0.07% to 1.57 ± 0.33% per min ($P < 0.005$). Repeated exposure to EA at 15 min intervals produced tachyphylaxis. The tachyphylaxis was not crossed with ouabain (5 μg/ml). Desipramine (1 μM) did not prevent the effect of EA. Removal of calcium from the incubation medium and increasing the magnesium concentration to 20 mM did not prevent the effect of EA.

It is concluded that EA releases noradrenaline from guinea-pig vas deferens by a mechanism different from that of tyramine and ouabain. The results suggest that the mechanism of action is calcium independent.

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