# SENSITIVITY OF CATECHOL-INDUCED SENSORY MYOCLONIC JERKS TO VALPROATE AND DIAZEPAM

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Recently, spontaneous convulsions induced by relatively high doses of catechol have been shown to be decreased by valproate and diazepam (Dawson & Dewhurst, 1984). Lower doses of catechol induce a central excitatory state in which myoclonic jerks can be evoked by a variety of sensory stimuli: for example electrical stimulation at the wrist evokes an electromyographic (EMG) response in forelimb flexor and extensor muscles, which typically consists of three temporally distinct components (M1, M2, M3). Each of these reflects activation of a different reflex pathway (Angel & Lemon, 1973; Dewhurst, 1984) and they are absent in the non-catechol treated anaesthetised animal. This work examines the effects of valproate and diazepam on catechol-induced sensory myoclonic convulsions.

Urethane-anaesthetised rats were infused with catechol (2.5mg $kg^{-1}$ $min^{-1}$ i.v.). EMG records were taken from flexor carpii and cutaneous afferents were stimulated electrically (50 μsec; 10-20V; 0.17Hz). Test drugs were administered after a suitable control period and their effects evaluated by measuring the probability of occurrence of each component of the EMG before and after drug administration. Results are expressed as the mean % difference in probability between control and test periods and are shown in Table 1.

Table 1 Effects of valproate and diazepam on EMG responses evoked during catechol infusion

| Drug | Dose mg $kg^{-1}$ i.v. | Mean % Difference ± s.d. M1 | M2 | M3 | n |
|---|---|---|---|---|---|
| valproate | 100 | no effect | -15.7 ± 15.3* | -61.3 ± 21.7* | 8 |
| | 250 | -20.7 ± 19.7 | -48.8 ± 38.4* | -84.4 ± 6.7* | 5 |
| diazepam | 1 | no effect | -41.3 ± 30.6* | -60 ± 12.9* | 5 |

* significantly different at 5% level (paired t-test)

The effects of both drugs were apparent within 5 min of administration and partial recovery occurred 30-40 min later.

Since M1 is probably a propriospinal reflex (Angel & Lemon, 1973) the actions of both anticonvulsants must be supraspinal and may be related to their facilitation of GABA-mediated inhibition (McDonald & Bergey, 1979; Costa et al, 1975). However preliminary experiments indicate that elevation of GABA levels by amino-oxyacetic acid has no effect on the catechol-induced EMG whereas sub-convulsive doses of picrotoxin increase the probability of M2 and M3.

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# MONOAMINE DEPLETION FACILITATES LEPTAZOL-INDUCED TONIC SEIZURES

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Many literature reports describe a proconvulsant effect of monoamine-depleting drugs. For example, reserpine pretreatment lowers the threshold for electrically-induced seizures (eg. Chen et al., 1954) and facilitates audiogenic seizures (Jobe et al., 1973). Similarly, the seizure response to leptazol also appears to be enhanced by prior administration of reserpine (Gross and Ferrendelli, 1982). Here we report the effects of monoamine depletion on seizures induced by a slow intravenous infusion of leptazol in mice.

Male Olac MFI mice (28-34g) were used in all experiments. A solution of leptazol (12 $mg.ml^{-1}$ in isotonic saline) was infused at a rate of 0.15 $ml.min^{-1}$ into a tail vein. The times to onset of clonic and tonic seizures were measured and seizure thresholds were calculated as $mg.kg^{-1}$ i.v. of leptazol. Drug or vehicle pretreatments (n=8-10) were assigned randomly.

The monoamine-depleting drugs tetrabenazine and reserpine were found to selectively decrease the threshold for leptazol-induced tonic seizures. The administration of 50 $mg.kg^{-1}$ s.c. tetrabenazine 2h before leptazol resulted in a decrease in the tonic seizure threshold to 49% of the control value ($p < 0.001$), without affecting the clonic seizure threshold. Reserpine (0.1-1.0 $mg.kg^{-1}$ s.c. administered 18h before leptazol) also reduced the dose of leptazol required to induce tonic convulsions (to 57% of control at 1 $mg.kg^{-1}$; $p < 0.001$) with no significant effect on the clonic seizure threshold. In subsequent experiments, reserpine (at a dose of 1 $mg.kg^{-1}$ s.c.) was occasionally found to increase the clonic seizure threshold significantly. This effect appeared to be a result of drug-induced hypothermia since it was completely abolished by maintaining the animals at normal body temperature. In contrast, the proconvulsant effect of reserpine on tonic seizures was not influenced by this treatment.

Selective 5-HT depletion using p-chlorophenylalanine (500 $mg.kg^{-1}$ p.o. administered 54h and 30 h before leptazol) had no effect on seizure thresholds whereas α-methylp-tyrosine (400 $mg.kg^{-1}$ and 100 $mg.kg^{-1}$ i.p. at 5h and 1h respectively before leptazol infusion) significantly reduced the tonic seizure threshold to 82% ($p < 0.05$) of control. The dopamine-β-hydroxylase inhibitor, FLA-63, was also found to be proconvulsant, decreasing both clonic and tonic seizure thresholds to 82% ($p < 0.01$) and 52% ($p < 0.001$) respectively of control values.

In an examination of the effects of several drugs on seizure thresholds in control and reserpinized animals (1 $mg.kg^{-1}$ s.c. reserpine, 18-20h before leptazol) the only treatment which reversed the proconvulsant action of reserpine was tranylcypromine (20 $mg.kg^{-1}$ i.p. 4h before leptazol). Other monoamine oxidase inhibitors (pargyline and nialamide) were ineffective. The following drugs also failed to restore normal tonic seizure thresholds in reserpinised animals: L-DOPA, L-5-HTP, d-amphetamine, clonidine, salbutamol and protriptyline.

Our results indicate that catecholamines are involved in the determination of the tonic seizure threshold, possibly suggesting that they can influence the rate of seizure propagation throughout the CNS, rather than seizure initiation. In this regard monoamine depleting drugs are distinguished from other proconvulsant compounds (eg. GABA antagonists and benzodiazepine contragonists) which markedly reduce the clonic seizure threshold.

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# ANTICONVULSANT ACTION OF LAMOTRIGINE, PHENYTOIN AND PHENOBARBITONE ON ELECTRICALLY-INDUCED AFTER-DISCHARGE

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Lamotrigine [3,5-diamino-6-(2,3,-dichlorophenyl)-1,2,4-trazine], a novel anticonvulsant, is one of the most potent, orally active, long duration compounds yet tested in the maximum electroshock (MES) test in mice and rats. (Miller et al, 1984).

In order to demonstrate an anticonvulsant effect of lamotrigine in the dog and marmoset, species used in toxicity and cardiovascular studies, the effects of lamotrigine were studied on the durations of after-discharges evoked by focal electrical stimulation of the hippocampus of the dog and the cortex of the marmoset. Similar studies were undertaken using the rat (hippocampal stimulation) to provide a comparison with the results obtained in a standard anticonvulsant test (maximum electroshock) in this species. All experiments were performed on halothane anaesthetised animals. The known anticonvulsants phenytoin and phenobarbitone were included for comparison.

Lamotrigine dose dependently reduced the duration of after-discharge in rat, dog and marmoset. (Table 1). Lamotrigine was approximately 2x more potent than phenytoin in the dog and approximately 3 to 4x more potent than phenobarbitone in both rats and dogs. Phenytoin was ineffective in the rat at sub-lethal doses (<40mg/kg). In limited studies in marmosets both lamotrigine and phenytoin (both at 5 to 15mg$kg^{-1}$) reduced or abolished after-discharges.

Table 1. Doses (mg $kg^{-1}$ i.v.) to reduce mean after-discharge duration by 50% in the rat and dog.

| Drug | Species | $ED_{50}$ mg $kg^{-1}$ | (95% fid. limits) |
|---|---|---|---|
| Lamotrigine | Rat | 11.7 | (8.7-16.6) |
| | Dog | 4.5 | (3.2-6.3) |
| Phenobarbitone | Rat | 32.0 | (21.3-59.5) |
| | Dog | 16.1 | (9.6-29.2) |
| Phenytoin | Dog | 7.1 | (4.1-13.8) |

Lamotrigine was a potent anticonvulsant in rat, dog and marmoset in an after-discharge model of partial (focal) seizures. Anticonvulsant doses of lamotrigine in this model in the rat were higher than those in the MES test. Similar potency ratios with phenobarbitone and phenytoin were obtained to those determined in the maximal electroshock test in mice and rats. The results of the present studies suggest that lamotrigine may be of value clinically for partial seizures in addition to its predicted usefulness for generalised seizures. (R.J.Lamb et al, This meeting).

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## 367P

# EFFECT OF INTRANIGRAL 2-AMINO-7-PHOSPHONOHEPTANOATE AND N-METHYL-D-ASPARTATE ON SEIZURES PRODUCED BY PILOCARPINE IN RATS

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A role for the substantia nigra in controlling the spread and generalization of seizures is indicated by experiments in rodents (Iadarola & Gale, 1982; McNamara et al, 1984). GABAergic mechanisms in the substantia nigra pars reticulata (SNR) influence the propagation of cortical epileptiform activity (Kaniff et al, 1983). Muscimol and γ-vinyl-GABA given intranigrally protect rats from electrically- and chemically-induced seizures (Iadarola & Gale, 1982). The anticonvulsant effect of GABA agonists within the substantia nigra may be due to a shift in the balance between excitation and inhibition. This suggests the possibility of an anti-convulsant effect within the SNR of drugs inhibiting excitatory amino acid-mediated neurotransmission. We report the action of 2-amino-7-phosphonoheptanoate (AP7) and N-methyl-D-aspartate (NMDA) in different parts of the substantia nigra in the pilocarpine model of limbic epilepsy in rats (Turski et al, 1983).

Adult male Wistar rats, 220-260 g, were stereotaxically implanted under pento-barbitone anesthesia (50 mg/kg; i.p.) with permanent guide cannulae directed towards the SNR (AP 1.6; L ± 1.9; V - 2.5), SNC (AP 1.6; L ± 1.9; V - 1.7), or dorsal striatum (AP 8.6; L ± 1.4; V + 1.0) (König & Klippel, 1963). For depth recordings (Beckman model RM polygraph), bipolar wire electrodes were placed into the amygdala (AP 4.9; L 4.0; V - 3.0) and hippocampus (AP 4.0; L 2.6; V + 1.7) (König and Klippel, 1963). Surface recordings were made fron jeweler screws positioned over the occipital cortex and referred to an indifferent electrode over the frontal sinus. Seven days after surgery, bilateral microinjections of (a) AP7 0.0001, 0.0005, 0.001, 0.005 and 0.01 umol, (b) NMDA 0.0005, 0.001, 0.002 and 0.005 umol or (c) saline were made into the SNR in a volume of 0.5 ul over a period of 5 min. AP7 0.005 umol was given into the SNC and into the dorsal part of the striatum. Pilocarpine HCL 100 or 380, mg/kg (Sigma; PIL) was administered i.p. N-methyl-scopolamine nitrate (Sigma) was given s.c. 30 min prior to PIL. AP7 and NMDA were given intranigrally or intrastriatally 15 min prior to PIL. The brains were processed for light microscopy 1-3, 5-7 or 21-30 days after micro-injection.

In rats pretreated with NMDA ($ED_{50}$ = 0.0015 umol ± 0.0011-0.002), a non-convulsant dose of PIL, 100 mg/kg, resulted in motor limbic seizures, which rapidly developed into status epilepticus. EEG monitoring showed progressive evolution of seizure activity with initial high voltage fast activity followed by high voltage spiking and electrographic seizures. Morphological analysis revealed widespread damage to the hippocampus, thalamus, amygdala, olfactory cortex, substantia nigra and neocortex. AP7 ($ED_{50}$ = 0.0007 umol ± 0.0004 - 0.0011), blocked the appearance of behavioural and electrographic seizures produced by PIL, 380 mg/kg, and prevented the occurrence of brain damage. AP7, 0.005 umol, when injected into the SNC or dorsal striatum did not antagonize seizures produced by PIL, 380 mg/kg.

The results indicate that excitatory neurotransmission in the SNR modulates the threshold for limbic convulsions produced by PIL in rats.

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# REGIONAL AMINO ACID LEVELS IN AUDIOGENIC SEIZURE SUSCEPTIBLE RATS DURING CONTROL AND SEIZURE CONDITIONS

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The excitatory amino acid antagonist, 2-amino-7-phosphonoheptanoic acid (2-APH) inhibits sound-induced seizures in AGS-susceptible (a Sprague-Dawley (SD) derived strain, University of Arizona) rats following systemic or focal administration into the inferior colliculus (IC) (Faingold et al, 1984). Amino acid transmitter alterations reported for this seizure-susceptible strain include reduced taurine uptake and synaptosomal taurine content (Bonhaus & Huxtable, 1983; Bonhaus et al, 1984), reduced GABA activation of benzodiazepine binding (Tacke & Braestrup, 1984), and increased number of GABA neurons in the IC (Roberts et al, 1984).

We have therefore determined regional amino acid levels in AGS-susceptible and resistant SD rats during control conditions and sound-induced seizures, tonic phase Seizures in AGS-susceptible rats were triggered by a mixed frequency sound of 109 dB intensity. The animals were killed by microwave irradiation during the tonic phase (32 sec after sound exposure). Additional groups (n=5) of susceptible AGS and resistant SD rats were killed by microwave irradiation during control conditions. Cortex (CX), cerebellum (CB), hippocampus (Hipp), striatum (Str), substantia nigra (SN), inferior and superior collicula (IC and SC) and reticular formation (RT) were extracted and analyzed for amino acid content by HPLC.

During control conditions, the aspartate levels in the AGS susceptible strain are 24-33% reduced in Hipp, Str, SN and IC compared to corresponding regions in the resistant SD strain. Glutamate levels are reduced 18-27% in Hipp and Str. There is a general 28-47% reduction in glutamine levels in all regions except CX. Glycine level is reduced (23%) in Hipp, taurine level is reduced (14%) in Str., and GABA level is increased (14%) in Hipp.

During the tonic phase of sound-induced seizures in AGS-susceptible rats there is a 57% increase in aspartate level in IC compared to corresponding AGS control level. Glutamate levels in CB and RF increase 21-32% during seizure. Glutamine levels are not affected by seizures in any region. Glycine levels increase 21-29% in Hipp and Str. Taurine levels increase 10-15% in CB and Str., and the GABA level decreases 27% in CX.

In conclusion, the previously shown (Faingold et al, 1984) 2-APH protection against sound-induced seizures in AGS-susceptible rats suggests an involvement of IC excitatory amino acids in seizure initiation. The observed rise in IC aspartate level during seizures and abnormalities in regional aspartate and glutamate levels support this suggestion. The uniform reduction in glutamine levels in most regions may also indicate altered glutamate transmission in AGS-susceptible rats. No abnormality in IC GABA level is observed, nor any general reduction in regional tau levels.

Supported by the MRC, Southern Illinois University and the Burroughs Wellcome Fund.

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369P

# ADRENOCEPTOR NUMBERS INCREASE AFTER KINDLING WITH THE BENZODIAZEPINE RECEPTOR LIGAND FG 7142

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Twenty four hours after electrical kindling in the rat there is a reduction in the number of cortical adrenoceptors. (Jefferys & Stanford 1984). While changes in $\alpha_2$ and $\beta$-adrenoceptor binding in the olfactory cortex were transient the reduction in $\beta$-binding in the remaining neocortex was persistent and may be associated with the reduced seizure threshold caused by kindling. Repeated administration of the benzodiazepine contragonist FG 7142 caused an increase in sensitivity to this compound in mice resulting in the production of seizures. This "chemical kindling" persists for at least one month after cessation of treatment (Little, Nutt & Taylor 1984). We have therefore examined the effects of chronic FG 7142 administration on adrenoceptor binding in the cerebral cortex of mice. Male CD1 (Charles Rivers) mice (30-35g) were given 40 mg $kg^{-1}$ FG 7142 i.p. once daily for 12 days. This schedule caused seizures in 50% of animals by the twelfth day. Control mice received vehicle injections (Tween 80, 1 drop in 10 ml distilled water 10 ml $kg^{-1}$). One week after the last injection the animals were killed by cervical dislocation, and the whole cortices removed. Preparation of membranes and binding protocol was as described previously (Jefferys & Stanford 1984). Control and FG 7142 treated tissues were assayed in pairs. Two batches of animals were studied (n values between 7 and 10 in each control and experimental group). Both batches gave the same results, which are pooled in the following table:

| | $^3$H-Clonidine ($\alpha_2$) | | | $^3$H-Dihydroalprenolol ($\beta$) | | |
|---|---|---|---|---|---|---|
| | Kd. | $B_{max}$ | | Kd. | $B_{max}$ | |
| Control | 1.08 ± 0.30 | 109 ± 8 | (14) | 0.39 ± 0.05 | 104 ± 8 | (16) |
| FG 7142 | 0.83 ± 0.14 | 120 ± 8* | (16) | 0.44 ± 0.06* | 117 ± 7* | (18) |

Values show mean ± s.e.m. $B_{max}$ (p. Moles $g^{-1}$ protein) and Kd (nM).
*P < 0.05 (matched-paired t-test). Numbers in parentheses indicate n values.

The rise in adrenoceptor binding after FG 7142 kindling contrasts with the down regulation seen after electrical kindling. Although species differences cannot be excluded the data suggest that adrenoceptor changes are not the cause of the convulsions seen after kindling. In view of the changes in adrenoceptor numbers associated with adaptation to stress (Stanford, Fillenz & Ryan 1984), it is possible that the up-regulation seen here after chronic FG 7142 may be related to the persistent increase in anxiety-related behaviour caused by this treatment (Jeevanjee, Little, Nicholass & Nutt, in press).

We thank Ferrosan (Denmark) for supplying FG 7142.

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# ANTICONVULSANT ACTION OF γ-D-GLUTAMYLAMINOMETHYLSULPHONIC ACID IN MICE

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Compounds that selectively block N-methyl-D-aspartic acid (NMDA)-induced excitation show anticonvulsant activity against sound-induced convulsions in genetically seizure prone DBA/2 mice, against a wide range of chemically-induced seizures in Swiss S mice, and against photically-induced seizures in baboons (Meldrum & Chapman, 1983). γ-D-Glutamylaminomethylsulphonic acid (GAMS) is an antagonist which in frog and cat spinal cord blocks excitation produced by kainate (KA) and quisqualate (QA) more effectively than responses induced by NMDA (Davies et al, 1982). The main objective of the present study was to determine the anticonvulsant spectrum of stereoisomers of γ-GAMS against a range of agonists with different selectivities for excitatory amino acid receptor subtypes in order to assess the specificity of protective action of these compounds in vivo.

Adult male, albino Swiss S mice, 18-20 g in weight, were used in groups of 7-10 animals. NMDA (Tocris), KA (Sigma), QA (Sigma), L-glutamic acid (L-GLU) (Sigma). Quinolinic acid (QUIN) (Sigma), D-homocysteine sulphinic acid (D-HSA) (Tocris) and γ-D- or γ-L-GAMS (Tocris) were brought into solution with minimum quantity of 1N NaOH, and made up to the final volume with 0.67 mM sodium phosphate buffer. All drugs were administered in a volume of 5 µl into the lateral brain ventricle of mice according to Herman (1975). The animals were subsequently observed for 30 min for the occurrence of clonic or clonic/tonic seizures. $CD_{50}$ values for myoclonic seizures (and their 95% confidence limits) were estimated from probit-log dosage regression curves. Relative potency ratios were determined for each oonvulsant by comparison of the $CD_{50}$ values for myoclonic seizures for administration of the convulsant alone, and in the presence of γ-D- or γ-L-GAMS, 1.0 µmol (Table 1). The data were statistically analysed according to Litchfield & Wilcoxon (1949).

Table 1 Protective efficacy of stereoisomers of GAMS against convulsant action of excitatory amino acid agonists in mice.

| Treatment | None | γ-D-GAMS | | γ-L-GAMS | |
|---|---|---|---|---|---|
| | $CD_{50}$ (µmol) | $CD_{50}$ (µmol) | Potency ratio | $CD_{50}$ (µmol) | Potency ratio |
| KA | 0.0003 (0.00017-0.00053) | 0.0272 (0.0225-0.0329)$^{+++}$ | 90.7 (49.3-164.5) | 0.002 (0.0014-0.0027)$^{+++}$ | 6.7 ( 3.4- 12.6) |
| QA | 0.0243 (0.0158 -0.0372 ) | 0.893 (0.4643-1.7185)$^{+++}$ | 36.7 (16.8- 80.0) | 0.0535 (0.0375-0.0765)$^{+}$ | 2.2 ( 1.3- 3.9) |
| NMDA | 0.00015(0.00009-0.00024) | 0.0044 (0.0029-0.0067)$^{+++}$ | 29.3 (14.8- 57.9) | 0.00019(0.00013-0.00027) | 1.3 ( 0.7- 2.3) |
| D-HSA | 0.0221 (0.0146 -0.0335 ) | 0.641 (0.4837-0.8481)$^{+++}$ | 29.0 (17.6- 47.9) | 0.043 (0.0296-0.0625) | 1.9 ( 0.9- 3.8) |
| QUIN | 0.01 (0.0066 -0.0151 ) | 0.213 (0.155 -0.2945)$^{+++}$ | 21.3 (12.7- 30.1) | 0.0158 (0.0128-0.0195) | 1.6 ( 1.0- 2.5) |
| L-GLU | 1.255 (1.025 -1.537 ) | 2.433 (1.662 -3.561 )$^{+}$ | 1.9 ( 1.3- 2.9) | 1.089 (0.758 -1.565 ) | 0.9 ( 0.6- 1.3) |

$^{+}P<0.05$; $^{+++}P<0.001$

The present study confirms at the supraspinal level the preferential kainate antagonist action of γ-D-GAMS detected in electrophysiological experiments on spinal cord neurons (Davies et al, 1982). On the other hands, our results demonstrate that γ-D-GAMS does not differentiate between quisqualate and NMDA responses. The high potency in protecting animals from seizures induced by kainic acid suggests the use of γ-D-GAMS as an antagonist of kainate-type responses in vivo.

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## 371P

# EFFECTS OF THE ANTICONVULSANT DRUG BECLAMIDE ON BEHAVIOURAL MODELS OF CENTRAL MONOAMINERGIC SYSTEMS

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Beclamide (N-benzyl-β-chlorpropionamide) is an anticonvulsant drug used to control behavioural disorders associated with manifest or latent epilepsy in children (Beley et al. 1962). Little information is currently available on the effects of this compound on central neurotransmitter systems. The present study examines the effects of Beclamide on models of central 5-hydroxytryptamine (5-HT) and dopamine (DA) systems.

Administration of 5-hydroxytryptophan (5-HTP) (100mg/kg i.p.) to animals pre-treated with pargyline (50mg/kg i.p.) results in a 5-HT dependent behavioural response consisting of Straub tail, hind limb abduction, lateral head-weaving and reciprocal forepaw treading in rats and of rapid head twitches in mice (Jacobs, 1976). Each component of the behavioural syndrome in rats was rated individually on a 0-3 scale (0=absent, 1=occasional, 2=frequent, 3=continuous) every 5 min. for 30 min following 5-HTP administration in Beclamide (50mg/kg i.p.) or saline pretreated animals. Head twitches in mice were counted similarly. The effect of Beclamide (50mg/kg i.p.) on locomotor activity stimulated by administering 3,4-dihydroxyphenylalanine (DOPA) (150mg/kg i.p.) to pargyline (50mg/kg i.p.) treated rats was measured in a 25cm x 46cm photocell cage. All experiments were performed with the observer unaware of the prior treatment of the animals.

Beclamide significantly reduced the behavioural syndrome in rats induced by 5-HTP and pargyline by 24% ($P<0.05$, Mann Whitney U test). Rats treated with Beclamide (50 or 100mg/kg) did not display signs of 5-HT receptor activation. The total number of head twitches in mice over the 30 min experimental period was not altered by Beclamide administration, however, a trend towards attenuated head-twitching was observed which was significant between 5-10 min after 5-HTP administration ($P<0.05$, Student's t-test).

Locomotor activity induced by DOPA and pargyline was not affected by Beclamide. However, the locomotor activity of untreated rats or rats given DOPA alone was reduced (60% and 56% respectively, $P<0.05$, Student's t-test).

These experiments suggest that Beclamide may possess some anti-5-HT activity and in addition that it reduces spontaneous, but not DOPA/pargyline induced locomotor activity. The relationship between these effects and the therapeutic effects of Beclamide remain to be established.

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# EFFECT OF VALPROATE ON STRIATAL ASCORBIC ACID, DOPAC AND 5-HIAA IN RATS AS MEASURED BY VOLTAMMETRY

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Sodium valproate (VPA) is an antiepileptic drug in wide clinical use. Although VPA increases brain concentrations of gamma-aminobutyric acid, it also modifies serotoninergic and dopaminergic activities in the brain, as indicated by post mortem biochemical analyses (Horton et al, 1977). Differential pulse voltammetry (DPV) is a technique which enables the continuous analysis of extracellular (rather than whole tissue) concentrations of ascorbic acid (AA) and 3,4-dihydroxyphenylacetic acid (DOPAC) (Gonon et al, 1980), or 5-hydroxyindole acetic acid (5-HIAA) (Crespi et al, 1983) in brain areas in vivo. We have recently improved this technique to enable the simultaneous quantification of these three acids with a single carbon fibre electrode in both anaesthetised and freely moving rats (Crespi et al, 1985).We have now employed this technique to study the effects of VPA on extracellular concentrations of these compounds in the rat striatum.

Initial studies in rats anaesthetised with chloral hydrate (400 mg/kg i.p.), phenobarbitone (60 mg/kg i.p.) or pentobarbitone (50 mg/kg i.p.) showed no significant changes in extracellular AA and DOPAC after injection of VPA (400 mg/kg i.p.). However, a slight but statistically significant reduction of 5-HIAA was observed (-14 % at 2.5 h, $p < 0.05$). Since VPA increased whole brain 5-HIAA in unanaesthetised rats (Horton et al, 1977), we repeated our studies in unanaesthetised, freely-moving animals. Under these conditions, the same dose of VPA markedly increased extracellular 5-HIAA levels (+ 30 % at 1h, $p < 0.05$ ; + 31 % at 2.5h, $p < 0.01$). In the same animals, VPA significantly reduced extracellular DOPAC (- 48 % at 1h, $p < 0.01$ ; - 84 % at 2.5h, $p < 0.01$). Extracellular AA levels were also reduced (- 48 % at 1h, $p < 0.01$ ; - 80 % at 2.5h, $p < 0.01$). This effect of AA may be related to the reduction of brain aspartate produced by VPA (Schechter et al, 1978), as this excitatory amino acid appears to release AA from synaptosomes (Fillenz and Grunewald, 1983). These data indicate that anaesthesia abolishes the effects of VPA on extracellular AA and DOPAC, and reverses the drug's effect of 5-HIAA. This supports the observation that anaesthesia may alter the response of cerebral neurones to various pharmacological agents (Trulson, 1984). Caution may thus be required in the interpretation of studies of the effects of VPA in anaesthetised animals, as the results obtained may not always reflect the situation in the absence of anaesthesia.

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# ABSENCE OF UPTAKE AND BINDING OF RADIOLABELLED QUINOLINIC ACID IN RAT BRAIN

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Quinolinic acid is an agonist at N-methyl-D,L-aspartate (NMDLA) receptors (Stone & Perkins, 1981) and in addition possesses kainate-like neurotoxic properties (Schwarcz & Kohler, 1983). We have now obtained radiolabelled quinolinic acid and sought the existence of uptake and binding processes.

(i) uptake: 1 μCi of 4-($^3$H)-quinolinic acid was incubated with each 400μm thick slice (approx. 4mg) of cortex or cerebellum prepared from adult male rats. After 30 minutes the slices were separated by filtration, washed and the tritium content determined by liquid scintillation spectrometry.

(ii) binding: Whole brain homogenates were prepared from adult male rats in 20 vol of ice cold buffer. The homogenate was first centrifuged at 4000g for 15 min and the pellet discarded, and then at 20,000g for 10 mins and the pellet resuspended in buffer. This was repeated three times. Binding was examined in Krebs bicarbonate medium and 50 mM Tris-HCl (pH 7.2). 800 μl portions (1 mg/ml) of the washed membrane fraction were added to 200 μl buffer containing 0.1 μM tritiated quinolinic acid (1 μCi). Non-specific binding was determined in the presence of 1 mM unlabelled quinolinate and following a 60 min incubation at 0°, 20° and 34°C the reaction was terminated by filtration and the filters washed with 20 mls of buffer.

(i) uptake: the results are shown in the following table as mean ± s.e.m. for 4 experiments, as c.p.m. per slice.

| Quinolinate conc. | | 100μM | 40μM |
|---|---|---|---|
| Cerebellum | 34°C | - | 1559±92 |
| | 0°C | - | 1361±32 |
| Cortex | 34°C | 1507±122 | 1589±172 |
| | 0°C | 1308±106 | 1556±70 |

Performing the experiments in the presence or absence of calcium or of magnesium, or adding the receptor antagonist kynurenic acid (100μM) did not result in any significant difference in the tissue counts from control values.

(ii) binding: no specific binding was detectable in either buffer under any of the conditions tested.

Clearly no active uptake system for quinolinic acid is present in the regions tested. These results complement those of Foster et al. (1984) who failed to detect quinolinate uptake into synaptosomes from striatum or hippocampus. In addition, like NMDA, quinolinate does not appear to bind specifically to brain membranes. These findings suggest that quinolinic acid does not resemble classical neurotransmitters in its interaction with neuronal systems.

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We thank New England Nuclear for the labelled quinolinic acid.

# DO L-GLUTAMATE BINDING SITES ON HUMAN ERYTHROCYTE PLASMA MEMBRANES PROVIDE A MODEL FOR CNS EXCITATORY AMINO ACID RECEPTORS?

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L-glutamate and L-aspartate are considered to be major excitatory transmitters in the mammalian CNS. Their receptors have been characterised by both neurochemical and neuropharmacological techniques, including the binding of L-$^3$H-glutamate and L-$^3$H-aspartate (Foster & Roberts, 1978; Sharif & Roberts, 1981)(For a review, see Foster & Fagg, 1984). Since possible abnormalities in excitatory amino acid function have been implicated in several neurological diseases, the aim of this study was to investigate whether glutamate receptors (in common with some other transmitter receptor systems) are expressed on human erythrocyte plasma membranes.Such a finding could greatly facilitate their study in neurodegenerative disorders.

Erythrocytes isolated from time-expired whole blood provided by the transfusion service, were washed, and then lysed in 5 mM Hepes buffer (pH 7.6). Erythrocyte membranes were pelleted by centrifugation and then washed further. The final membrane pellet was resuspended in 50 mM Hepes buffer containing 2.5 mM $CaCl_2$. For the binding assay, membrane suspensions were incubated with L-$^3$H-glutamate (39 Ci/mmol), usually at a final concentration of 10.7 nM for 30 min at ambient temperature. Specific binding was defined by inclusion of 100 $\mu$M unlabelled L-glutamate. Assays were terminated by centrifugation at 50,000 g. The pellets were washed superficially and bound radioactivity determined by liquid scintillation counting.

L-$^3$H-glutamate was bound avidly, to apparently a single population of sites of medium affinity ($K_D$= 35.6 $\pm$ 6.9 $\mu$M and $B_{max}$= 3133 $\pm$ 483 pmol/ mg protein). Binding occurred rapidly and was linear over a rather restricted protein concentration range (up to 125 $\mu$g/assay). There was little temperature dependence, although incubation at 50$^o$ abolished binding. Binding showed a sharp pH optimum, around pH 7. A number of acidic amino acids and their analogues, including L- and D-aspartate, DL-homocysteate, N-methyl-DL-aspartate, L-glutamate diethylester, DL-2-amino-4-phosphonobutyrate, threo-3-hydroxyaspartate and kainate, were ineffective or only very weakly active as inhibitors of binding at a concentration of 100 $\mu$M. Strikingly, only L-glutamate itself strongly inhibited binding ($IC_{50}$ approx. 0.2 $\mu$M).

The nature and significance of the high density of sites on human erythrocyte membranes to which L-glutamate binds is unclear. The pharmacology is unlike that found for either CNS binding sites, or sites for acidic amino acid uptake. L-glutamate is not readily taken up into erythrocytes.When needed, for example for glutathione synthesis, it is usually provided from the uptake and subsequent deamination of glutamine (Ellory et al., 1983). Thus, it would appear from this study that erythrocytes do not provide an appropriate model for the study of excitatory amino acid receptors.

This work was supported by grants from the S.E.R.C. and N.A.T.O.

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## 375P

# EFFECT OF PROLONGED TREATMENT OF PRIMARY AFFERENT C-FIBRES WITH MAXIMAL DOSES OF KAINATE

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The neurotoxicity of kainic acid (Coyle, 1983) is evident following treatment of in vitro spinal cord preparations. Prolonged treatment with maximal doses of kainate produces irreversible depolarization of motoneurones and complete loss of spontaneous and reflex activity recorded in ventral roots (Evans, 1980). The neurotoxic effect is attributed to prolonged depolarization and increase in conductance mediated by kainate receptors. Axons in general are believed to be insensitive to kainate (Coyle, 1983). However, it has been reported recently that the central ends of primary afferent C-fibres are depolarized by kainate (Evans, 1985). Thus it was of interest to see if the latter effect was associated with neurotoxicity to C-fibres.

Orthodromic C-fibre volleys, generated following ischiadic nerve stimulation, were recorded from L5 dorsal roots isolated from 4-8 day old rats. Preparations were tested initially with a brief application (5 min) of 10μM kainate in order to reveal kainate-sensitive C-fibre volleys. Treatment with 1mM kainate for 30-180 min (3 preparations) failed to irreversibly depress the kainate-sensitive component of C-fibre volleys following washout of 1mM kainate. Therefore it would appear that the depolarization of primary afferent C-fibres by kainate is not associated with a neurotoxic action. Such freedom from neurotoxicity may depend on a lack of kainate receptors on the dorsal root ganglion neurones which give rise to these C-fibres.

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# SELECTIVITY OF DOXAZOSIN FOR $\alpha_1$ ADRENOCEPTORS

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Doxazosin (1-(4-Amino,6,7,dimeth oxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-yl-carbonyl)piperazine) is a new alpha$_1$ adrenoceptor antagonist possessing a pharmacokinetic profile which allows 24 hr control of blood pressure after a single daily dose. The selectivity of doxazosin for alpha$_1$ as opposed to alpha$_2$ adrenoceptors both *in vitro* and *in vivo* is now reported.

In pentobarbitone anaesthetised dogs pretreated with desimipramine, doxazosin (10-500 µg/kg i.v.) reduced responses of the nictitating membrane to electrical stimulation of the vago-sympathetic-depressor nerve trunk (an alpha$_1$ adrenoceptor response) but had no effect on the chronotropic response of the heart to electrical stimulation of the ansa subclavia nerve. In contrast, the pre-junctional alpha$_2$ adrenoceptor antagonist activity of yohimbine (10-100 µg/kg i.v.) was manifest as a marked dose-related increase in both the heart rate and nictitating membrane responses. In chloralose anaesthetised cats, doxazosin 50-100 µg/kg i.v. inhibited pressor responses to injected phenylephrine but had no effect on pressor responses to injected alpha-methylnoradrenaline and angiotensin. The dose-response curves to phenylephrine were shifted to the right in a parallel fashion and doxazosin was some 6 times less potent than prazosin in this respect.

Thus doxazosin is a potent and selective antagonist of vascular alpha$_1$ adrenoceptors without effect on either pre-junctional or vascular post-junctional alpha$_2$ adrenoceptors. Studies in isolated tissues have also shown the lack of activity of doxazosin at alpha$_2$ adrenoceptors (Cambridge and Davey, 1980). The high degree of selectivity of doxazosin for alpha$_1$ sites was confirmed by ligand binding studies in rat brain membranes where the mean Ki values ($\pm$ SEM n = 6) for displacement of $^3$H-prazosin, $^3$H-rauwolscine and $^3$H-clonidine were 1.1 $\pm$ 0.2 nM, 200 $\pm$ 70 nM and $>$ 10µM respectively. The affinity of doxazosin for alpha$_1$ binding sites in the rat brain preparation was some 5 times less than that of prazosin (Ki 0.23 $\pm$ 0.02 nM).

Preliminary experiments using tritiated doxazosin in rat brain membranes indicate that it will provide a selective ligand for labelling alpha$_1$ adrenoceptors. Since the specific activity of tritiated doxazosin (53 Ci/mmol) is higher than that of tritiated prazosin (Amersham 10-30 Ci/mmol), it may provide a superior ligand for labelling and profiling peripheral adrenoceptors e.g. in vascular tissue.

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377P

# INHIBITION OF AGONIST-INDUCED $Ca^{2+}$ FLUX IN HUMAN NEUTROPHILS BY TUMOUR PROMOTING PHORBOL ESTERS

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The activation of human neutrophils by soluble and particulate stimuli is regulated by the actions and interactions of at least two second messenger molecules: cytosolic free calcium ($[Ca^{2+}]i$) and 1,2-diacylglycerol (DAG) (Di Virgilio et al, 1984) that are produced as a consequence of receptor-mediated phosphoinositide hydrolysis (Nishizuka, 1984). The former acts via stimulation of $Ca^{2+}$-calmodulin dependent protein kinase(s) and the latter via stimulation of protein kinase C. The effects of DAG on protein kinase C are mimicked by tumour-promoting phorbol esters e.g. 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (Castagna et al, 1982). Using TPA and human platelets we have recently shown that, besides mediating cellular activation, protein kinase C may also regulate agonist-induced $Ca^{2+}$ flux (MacIntyre et al, 1985). In the present study we examined the effects of TPA on changes in $[Ca^{2+}]i$ in human neutrophils elicited by the chemotactic tripeptide formylmethionylleucylphenylalanine (FMLP), leukotriene $B_4$ ($LTB_4$) and 1-O-hexadecyl-2-acetyl SN glycero-3-phosphorylcholine (platelet-activating factor, PAF).

Human neutrophils were isolated from the blood of healthy adult donors by gelatin sedimentation followed by hypotonic lysis of contaminating erythrocytes. For estimation of $[Ca^{2+}]i$ suspensions of neutrophils ($10^8$ cells/ml, 98% viable) in a modified Hanks balanced salt solution were loaded with quin 2-acetoxy-methylester essentially as described by White et al (1983), followed by separation from extraneous dye by centrifugation and resuspension in the same buffer at $10^7$ cells/ml. Changes in fluorescence were monitored in a Perkin-Elmer LS3 Fluorescence spectrometer at 37°C with excitation at 339nm and emission at 492nm.

Resting $[Ca^{2+}]i$ in neutrophils was 133±9nM (mean ± S.E., n = 12). FMLP (0.05-500 nM), $LTB_4$ (0.1-1000nM) and PAF (0.1-1000nM) induced a rapid, concentration-dependent elevation of $[Ca^{2+}]i$ to around 250-400nM in different experiments using neutrophils from different donors. Prior exposure of neutrophils to TPA (1-100nM; 2 min; 37°C) but not to DMSO (vehicle control) inhibited the elevation of $[Ca^{2+}]i$ induced by all three agonists.

Such inhibition of FMLP-, $LTB_4$-, and PAF-induced $Ca^{2+}$ flux in human neutrophils by TPA confirms and extends the observations of Lagast et al (1984) using FMLP. These results indicate that in neutrophils, as in platelets, protein kinase C may subserve a bidirectional role in regulating cellular activation.

This study was supported by the MRC, SERC and by the Medical Funds of the University of Glasgow. A.G.R. is an SERC scholar.

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# IN VITRO STUDIES ON THE ACETYLATION OF SULPHAMETHAZINE BY HUMAN WHOLE BLOOD

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The antibacterial drug sulphamethazine (SMZ) is polymorphically acetylated in vivo. The reaction is catalysed by N-acetyltransferase (NAT) and requires acetyl CoA as the acetyl group donor. The highest NAT activity is located in the liver although extrahepatic NAT sites have also been identified (Weber et al, 1980). Blood is one of the most accessible tissues for studying extrahepatic metabolism. However, studies on the acetylation of SMZ by blood obtained from several species, including man, have been hindered by the lack of a sensitive and specific analytical method for measurement of the acetylsulphamethazine (AcSMZ) produced.

We have developed an HPLC method which allows detection and reliable quantitation of AcSMZ. Using this assay, we have investigated some of the factors which affect the enzyme activity and obtained estimates for the kinetic parameters $V_{max}$ and $K_m$ of the enzyme. We have also studied variation of the enzyme activity within a small group of healthy volunteers to determine whether any relationship exists between the in vivo acetylator phenotype and the blood NAT activity in vitro.

Samples were prepared by adding 0.5 ml heparinised whole blood to 0.5 ml SMZ solution in phosphate buffer (pH 7.4, 33.3 mM) to produce final SMZ concentrations in the range 18 µM-1.44 mM. The tubes were then incubated for 24 hours at 37°C with rotary mixing. After extraction with ethyl acetate and evaporation of the solvent, the free and acetylated compounds were separated on an Altex Ultrasphere ODS (5 µm) column (250 mm, 4.6 mm i.d. stainless steel) with a mobile phase of acetonitrile/phosphate buffer, pH 7.4, 33.3 mM (15/85) at a flow rate of 1 ml $min^{-1}$. The compounds were detected using a variable wavelength UV detector set at 240 nm. Under these conditions, AcSMZ eluted before SMZ.

SMZ is acetylated by human whole blood but not by plasma. The temperature range 30-37°C was optimal for blood NAT. Heating at 50°C for 5 minutes reduced the enzyme activity by approximately 50%. A 10 fold molar excess of added acetyl CoA to an initial SMZ concentration of 180 µM approximately doubled the % acetylation.

In the absence of added acetyl CoA, the acetylation reaction is linear up to at least 7 hours. The apparent $V_{max}$ and $K_m$ values of blood NAT using SMZ as substrate were 86 pmoles $min^{-1}$ ml $blood^{-1}$ and 416 nmoles $ml^{-1}$. These values were estimated using the direct linear plot (Eisenthal and Cornish-Bowden, 1974). The estimates of $V_{max}$ and $K_m$ using the hyperbolic plot were 74 pmoles $min^{-1}$ ml $blood^{-1}$ and 334 nmoles $ml^{-1}$.

Inter-individual variation of blood NAT activity was studied using three SMZ concentrations (18 µM, 0.18 mM and 1.44 mM). Intra-individual variation of this activity over a ten month period was slight (CV <10%). The inter-individual study (n= 11) revealed an approximately two fold variation at each substrate level. Of the 11 subjects, 6 have been phenotyped for acetylator status. Differences in acetylator phenotype do not appear to be revealed by in vitro acetylating activity.

This method may enable better characterisation of blood NAT which may be important in assessing the contribution of extrahepatic NAT sites to the overall acetylation process in vivo.

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**379P**

# COMPARISON OF EFFECTS OF NIFEDIPINE AND NISOLDIPINE ON THE HUMAN ISOLATED UTERINE ARTERY PREPARATION

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Both nifedipine and nisoldipine are dihydropyridine calcium channel blockers. It has been shown that nisoldipine is more potent than nifedipine in inhibiting $K^+$-induced contraction in the rabbit aorta (Kazda et al., 1980; Schumann et al.,1975). In this study, we have investigated effects of nifedipine and nisoldipine on $K^+$- and noradrenaline-induced contraction in the humanuterine artery preparation.

Human uteri were obtained from a hospital right after hysterectomy. Uterine arteries were dissected and kept in ice-cold Krebs solution. Arterial rings about 0.5 cm long were suspended in Krebs solution, bubbled with 95% $O_2$ and 5% $CO_2$, and kept at 37°C. Resting tension about 2 g was chosen. Changes in tension were measured isometrically. Cumulative dose-response relationships were established.

The preparation contracted to $K^+$ and noradrenaline. 11,9- epoxymethane $PGH_2$, a thrombaxane $A_2$ mimetic, at 300 nM produced little effect.

A dose-response relationship to KC1 was established first. After washout, the tissues were equilibrated with nifedipine (1h) or nisoldipine (2h) and second dose-response relationship was then obtained. Further does-response relationships were obtained in the same way using higher concentration of the calcium channel blockers. $K^+$-induced contraction were inhibited by nifedipine and nisoldipine. The $IC_{50}$ values are 7.9 ($\pm$ 2.1) x $10^{-9}$ M (n = 8) for nifedipine and 7.2 ($\pm$ 1.2) x $10^{-9}$M (n = 12) for nisodlipine.

Nifedipine and nisoldipine were weak antagonists of noradrenaline on the human uterine artery preparation, nisoldipine being slightly more potent than nifedipine. This antagonism was further analysed in the following way: a fixed concentration of noradrenaline (10 µM) was added, kept for 5-10 min, then washed out and this cycle was repeated while nifedipine or nisoldipine was added in increasing concentrations. Both nifedipine and nisoldipine were able to inhibit about 40% of the control contraction but the remainder was resistant to them. Thus, the response to noradrenaline appeared to comprise two components -about 40% being sensitive to the dihydropyridine calcium channel blockers and about 60% not being sensitive. The $IC_{50}$ for inhibition of the dihydropyridine sensitive component comes out at surprisingly low values, i.e. 1.2 ($\pm$ 0.3) x $10^{-8}$ M (n = 9) for nisoldipine and 1.6 ($\pm$ 0.5) x $10^{-7}$M (n = 11) for nifedipine.

In $Ca^{++}$-free Krebs solution containing EDTA $10^{-3}$M, responses to KC1 were almost abolished, but noradrenaline was able to give 50% of control contraction.

Our results show that nifedipine and nisoldipine have similar potency in inhibiting $K^+$ -induced contraction in the human uterine artery preparation. Furthermore, noradrenaline manoeuvres both intracellular and extracellular calcium; for inhibiting the entry of extracellular calcium, nisoldipine show higher potency than nifedipine. In our opinion, if an agonist produces contraction by utilizing both extracellular and intracellular calcium, the calcium channel blocker-resistant component of contraction, i.e. contraction induced by release of intracellular calcium, should be subtracted, so that a correct assessment of the $IC_{50}$ value of a calcium channel blocker against the agonist can be made.

We are grateful to the Bayer U.K. Limited for financial support

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# OPIOIDS ACT ON μ RECEPTORS TO REDUCE DORSAL ROOT POTENTIAL AMPLITUDE IN RAT ISOLATED SPINAL CORD

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The effects of opioids have been studied on the dorsal root to dorsal root evoked potential (DRP) of the neonatal rat hemisected spinal cord in vitro. Applications of [D-Ala$^2$,Met$^5$]enkephalinamide (DAME) ($10^{-7}$-$10^{-5}$M), [D-Ala$^2$, D-Leu$^5$]enkephalin (DADL) ($10^{-7}$-$10^{-5}$M), Leu$^5$ enkephalin ($10^{-5}$-$2\times10^{-4}$M), Met$^5$ enkephalin ($10^{-5}$-$2\times10^{-4}$M), dynorphin 1-9 ($10^{-5}$-$2\times10^{-4}$M) and normorphine ($10^{-6}$-$5\times10^{-5}$M) produced dose-dependent depressions of the DRP. Maximal inhibitory effects were between -15 and -40% of control amplitude. These effects were antagonised by naloxone ($5\times10^{-8}$M) but not by the highly selective delta-opioid receptor antagonist ICI 174864 ($10^{-6}$M). The kappa agonists, U50488, tifluadom, ethylketocyclazocine (EKC) and bremazocine had only weak and inconsistent inhibitory effects on the DRP, causing <10% reduction of amplitude with very slow onset. Morphine had no inhibitory agonist effect on the DRP in these experiments. Instead, it had antagonist actions against the effective opioids, having an $IC_{50}$ of about $8.5\times10^{-7}$M (Fig 1). EKC, bremazocine and buprenorphine were also antagonists but tifluadom was not.

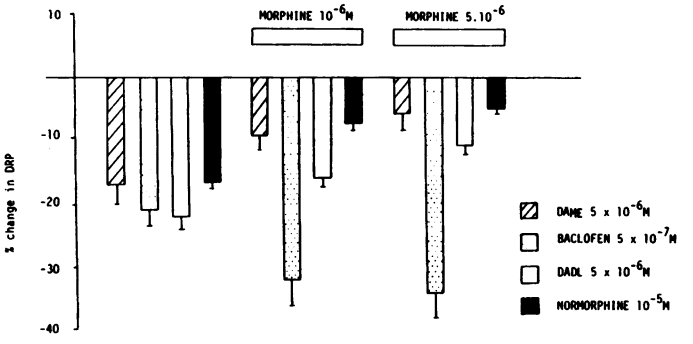


FIG 1: ANTAGONISM SHOWN BY MORPHINE HYDROCHLORIDE ON THE DEPRESSANT EFFECT OF OPIATES AND ENKEPHALINS ON THE DRP.

Morphine was applied for 20 mins before repeating the agonists. Each bar represents the mean percentage change in DRP ± SE of three different experiments. DAME, D-Ala$^2$Met$^5$-enkephalinamide. DADL, DAla$^2$DLeu$^5$Enkephalin.

This evidence indicates that the receptors involved in DRP inhibition in this preparation are of the mu opioid type. High intrinsic activity is required for effectiveness, and the receptor reserve is small. Thus, compounds with opioid partial agonist or mixed agonist-antagonist actions, which act as antagonists on the rat vas deferens preparation are also opioid antagonists on the rat DRP.

381P

# FENFLURAMINE-INDUCED HYPERTHERMIA - AN IN VIVO MODEL FOR THE EVALUATION OF DRUGS ACTIVE AT 5-$HT_2$ RECEPTORS

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Fenfluramine-induced hyperthermia in the rat involves the release of endogenous 5-HT from the hypothalamic thermoregulatory pathways (Cox, et al., 1982). This hyperthermic response has been claimed to be specifically antagonised by drugs which either block 5-HT receptors, inhibit fenfluramine uptake into 5-HT nerve endings or lower 5-HT stores (Frey, 1975; Sulpizio et al., 1978). We have now attempted to define the 5-HT receptor sub-type involved in this response using a number of 5-HT antagonists. Intraperitoneal injection of a standard dose of fenfluramine (15mg/kg i.p.) into groups of 8 Alderley Park SPF female rats (180-200g), maintained at an ambient temperature of approximately 28°C, caused a submaximal increase in core temperature of approximately 1°C. Core temperature was measured by means of a rectal thermistor probe inserted to a depth of 6cm. The dose of antagonist which reduced the response to fenfluramine by 50% ($ID_{50}$) was determined by linear regression from a log dose v % inhibition of response plot. The potency of a number of standard 5-HT antagonists using this experimental paradigm are presented in Table 1.

Table 1 Antagonism of fenfluramine-induced hyperthermia in the rat

| Antagonist | $ID_{50}$ μ moles/kg s.c. | (95% confidence limits) |
|---|---|---|
| Methergoline | 0.017 | (0.005 - 0.10) |
| Pizotifen | 0.03 | (0.003 - 0.14) |
| Cyproheptadine | 0.06 | (0.03 - 0.1) |
| Pirenperone | 0.08 | (0.03 - 0.1) |
| Methiothepin | 0.59 | (0.28 - 1.28) |
| Mianserin | 1.13 | (0.04 - 3.0) |
| Ketanserin | 0.9 | (0.4 - 2.0) |
| Cinanserin | 1.44 | (0.62 - 3.32) |
| Methysergide | 1.41 | (0.14 - 15.6) |
| Triflupromazine | 2.2 | (0.92 - 5.0) |
| Amitriptyline | 3.4 | (1.08 - 13.72) |
| Trazadone | 3.76 | (2.15 - 5.37) |
| MDL 72222 | 31.5 | (15.9 - 62.4) |

As can be seen from Table 1, all compounds with a high affinity for 5-$HT_2$ binding sites and the recently purported selective 5-$HT_2$ antagonists ketanserin and pirenperone (Leysen, et al., 1982) are potent inhibitors of fenfluramine-induced hyperthermia whereas, the neuronal (M) receptor antagonist MDL 72222 (Fozard, 1984) was the least active in this test. It has previously been reported that 5-HTP-induced head twitches in the mouse are mediated by 5-$HT_2$ receptors (Peroutka, et al., 1981). Thus we have measured the ability of the 5-HT antagonists shown in Table 1 to antagonise 5-HTP-induced head twitches and a positive correlation between antagonism of fenfluramine-induced hyperthermia and this test was obtained ($r=0.8$, $P < 0.0001$, $n=13$). Therefore these results suggest that antagonism of fenfluramine-induced hyperthermia in the rat provides a convenient quantitative in vivo model for the detection of 5-$HT_2$ antagonist activity.

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# κ-OPIOID DIURETIC EFFECTS OF TIFLUADOM MEDIATED IN THE PERIPHERY?

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Kappa opioid agonists have recently been shown to produce marked diuretic effects in normally hydrated rats (Leander 1983a, 1983b). This diuresis apparently involves a neurohypophyseal site of action leading to a suppression of plasma vasopressin levels (Miller, 1975). However, vasopressin has been recently identified in rat adrenal medulla (Ang and Jenkins, 1984) and evidence is now presented to show that the diuretic response to the recently described k-agonist tifluadom (Römer et al., 1982) may involve a peripheral site of action since it is abolished following bilateral adrenal demedullation in the rat. Male Alderley Park rats (~200g) underwent bilateral adrenal enucleation and were challenged three days after surgery with a standard diuretic dose of tifluadom (3.5mg/kg s.c.). In normal, conscious sham, saline loaded (2.5ml/100g, 0.9%) control rats (n=12), tifluadom induced a characteristic diuresis (24.3 ± 0.9ml) compared with saline controls (8.3 ± 0.7ml) 2h after dosing. However, following demedullation, no significant difference in urine output was apparent between the demedullated (8.7 ± 1.3ml) and saline controls (10.3 ± 3.0ml). This data indicates a link between the adrenal medulla and tifluadom-induced diuresis and hence a peripheral rather than a central site of action appears to be primarily involved in tifluadom-induced diuresis. Adrenal demedullation reduced adrenal gland catecholamine (adrenaline and noradrenaline) content by 99.5% and plasma adrenaline levels by 91%. However, adrenaline does not appear to play a role in tifluadom-induced diuresis since pretreatment with propranolol (1.0mg/kg s.c.) and idazoxan (0.2mg/kg s.c.) had no effect on the diuretic response in intact animals. Plasma corticosterone levels were not significantly affected, indicating that the adrenal cortex remained both intact and functional. Plasma vasopressin levels have yet to be measured.

Thus this study provides evidence for a peripheral site of action of tifluadom-induced diuresis in the rat. Further work is in progress to establish whether this action is unique to tifluadom.

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383P

# CONTRALATERAL TURNING INDUCED BY INTRASTRIATAL INJECTION OF SULPHATED CCK-8: ANTAGONISM BY CCK-27-32NH$_2$

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There is now growing evidence that the octapeptide derivative of cholecystokinin (CCK-8) plays an important role in the modulation of dopaminergic (DAergic) neurotransmissions within the forebrain (Morley, 1982). CCK-8 has been reported to be co-released with DA in the n. accumbens but not in the striatum. Although often contradictory, the available data suggest that DA-CCK-8 interactions are not similar and could even be opposite in nature in these two structures.

Using the technique of direct injection into the mouse striatum, we have investigated the turning induced by sulphated CCK-8 (CCK-8S), alone or in combination with the antagonists proglumide (Hsiao et al, 1984) and CCK-27-32NH$_2$ (Spanarkel et al, 1983), and with the neuroleptic haloperidol.

Female Swiss CD$_1$ mice (25-30 g, Charles River, France) were used. Direct intrastriatal injections of drugs, dissolved in a pH6 phosphate buffer, or in DMSO (proglumide), were made according to Bizière et al, 1985. For interaction studies, the two drugs were co-injected in the same solution, except for haloperidol. The number of complete turns was counted between 2 and 4, 8 and 10, 13 and 15 min after injection.

The vehicle-injected mice exhibited a weak, short-lasting ipsilateral turning (2-4 turns/6 min). CCK-8S induced contralateral turning with a threshold dose of 0.05 ng, but with a U-shapped dose-response curve (mean ± SEM nb. of turns/6 min - 0.01 ng : + 1.2 ± 1.1 ; 0.05 ng : - 6.1 ± 1.1 ; 0.1 ng : - 10.3 ± 1.8 ; 0.5 ng : - 14.7 ± 1.8 ; 1 ng : - 7.6 ± 1.3 ; 10 ng : - 4.5 ± 1.2). CCK-8NS (1 and 10 ng) was completely inactive.

The effect of CCK-8 S (0.5 ng) was dose-dependently antagonized by co-injection of CCK-27-32NH$_2$ (CCK-8S : - 14.5 ± 1.0 ; + CCK-27-32NH$_2$, 0.01 ng : -10.4 ± 0.7* ; + 0.1 ng : - 5.2 ± 0.4** ; + 1 ng : - 3.0 ± 0.4**). In these conditions, co-injected proglumide also antagonized CCK-8S (0.5 ng)-induced turning (CCK-8S : - 14.8 ± 1.4 ; + Prog., 100 ng : - 6.3 ± 1.0** ; + Prog., 1 µg : - 2.8 ± 0.8**). Proglumide thus appears to be about 1000 times less potent than CCK-27-32NH$_2$, on a ng basis (3000 times on a molar basis).

Finally, this effect of CCK-8S (0.5 ng) was dose-dependently antagonized by i.p. pretreatment with haloperidol (H) (CCK-8S : - 12 ± 1.6 ; + H 0.03 mg/kg : - 9.4 ± 1.2 ; + H 0.1 mg/kg : - 7.2 ± 0.9** ; + H 0.3 mg/kg : - 0.8 ± 0.4**).

In these experimental conditions, the DA agonist apomorphine (A) also induced a neuroleptic-sensitive contralateral turning (A 0.5 µg : - 13.5 ± 1.5 ; A + H 0.1 mg/kg, i.p. : - 2.4 ± 0.6**).

These data confirm a DA-CCK-8S interaction within the mouse striatum and suggest that, for this behaviour, CCK-8S stimulates DA-mediated neurotransmission. This would fit with previous reports showing that ICV CCK-8S induces a decrease in striatal DA turnover and down-regulates $^3$H-spiperone labeled binding sites (Mashal et al, 1983).

Moreover, these data show that CCK-27-32NH$_2$, in addition to its peripheral effects (Spanarkel et al, 1983), is also a very potent antagonist of central CCK-8S-mediated effects. In this respect, CCK-27-32NH$_2$ appears considerably more potent than proglumide.

* $p < 0.05$ ; ** $p < 0.01$ vs respective controls (Student's t test).

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# CM 40907, A NEW POTENTIAL ANTIEPILEPTIC DRUG WITH A BROAD SPECTRUM OF ANTICONVULSANT ACTIVITIES

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CM 40907, 3-(4'-hydroxy-piperidinyl)-6-(2-chlorophenyl)-pyridazine, is a new potent, and chemically original anticonvulsant drug which exhibits a broad spectrum of anticonvulsant activities in mice, rats and baboons, with few sedative side-effects. Female Swiss mice ($CD_1$, Charles River, France ; 19-23 g ), male Sprague Dawley rats (CD COBS, Charles River, France ; 200-300 g) and senegalese Papio-papio photoepileptic baboons (4-9 kg), were used in these studies. The effect of CM 40907, carbamazepine (CBZ) and sodium valproate (VPA) against seizures and death induced by maximal electroshock (MES), metrazol (105 mg/kg, i.p.), bicuculline (0.9 mg/kg, i.v.), picrotoxin (7 mg/kg, s.c.), 3-mercaptopropionic acid (3-MPA ; 60 mg/kg, s.c.) or strychnine (1 mg/kg, i.p.), were investigated in mice, as described by Chambon et al (1984). The antagonism of kindled amygdaloid seizures was assessed in rats according to Goddard et al (1969). In these rodent experiments, drugs or vehicle were administered orally (p.o.) 60 min before the convulsant challenge. The activity of the test drugs (p.o.) was also studied in the photoepileptic baboon according to Meldrum et al (1975). The motor sedative effect of CM 40907 was assessed in mice using the traction and rota-rod tests (Boissier et al, 1961).

Table 1

| | ED50's (mg/kg, p.o.) | | | | | | |
|---|---|---|---|---|---|---|---|
| Test Drugs | MES tonic | Metraz.[a] tonic | Bicuculline[b] tonic | Bicuculline death | Picrot.[a] tonic | 3-MPA tonic | Strych.[a] seiz.[b] |
| CM 40907 | 16 | 29 | 38 | 34 | 34 | 12 | > 100 |
| CBZ | 21 | 21 | 29 | 16 | 10 | 9 | 92 |
| VPA | 349 | 201 | 274 | 246 | > 400 | 181 | > 500 |

a Picrot. = picrotoxin ; Metraz. = metrazol ; Strych. = strychnine
b tonic = tonic seizures ; seiz. = tetanic seizures

As shown in Tab. 1, CM 40907 antagonized the convulsive and lethal effects of all the chemical convulsants but strychnine, and is also highly active against electroshock, in mice. In this respect, CM 40907 appeared nearly as active as CBZ and 10 times more active than VPA. In addition, CM 40907 antagonized kindled amygdaloid seizures in rats with a minimal effective dose of 20 mg/kg, p.o. ; the MED for CBZ was 10 mg/kg, p.o.. In the baboon, CM 40907 was more effective than CBZ. Thus, at 40 mg/kg, p.o., the former drug protected 3 out of 4 monkeys against photic seizures (mean duration : 3 h), whereas the latter drug elicited protection in only 1/4 monkeys (during 4 h). At 100 mg/kg, p.o., the protection induced by CM 40907 occurred in 4/4 animals up to 7 hours, whereas CBZ was active in half the animals (during 7 h). Finally, the oral ED50's for motor sedation in mice were 98-138 mg/kg for CM 40907, 95-115 mg/kg for CBZ, and 194-273 mg/kg for VPA, indicating that CM 40907 is weakly sedative. These data indicate that CM 40907 is a new potent broad-spectrum anticonvulsant drug inducing few sedative effects. This suggests a potential usefulness of CM 40907 in the treatment of various epileptic states.

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385P

# TURNING INDUCED BY INTRASTRIATAL INJECTIONS IN CONSCIOUS MICE: A USEFUL SCREENING MODEL FOR DOPAMINOMIMETIC DRUGS

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Among a wide range of behaviours, dopamine (DA) and DAmimetic drugs were reported to induce contralateral postures when injected unilaterally into the caudate nucleus or pallidum. This was done mainly in rats, and included induction of "postural assymetry" and/or "head-turning" (for review, see Pycock, 1980). We report here the induction of real "turning" behaviour after direct free-hand unilateral injection of DAergic drugs within the striatum of conscious mice.

Female Swiss $CD_1$ mice (25-30 g, Charles River) were used. Drugs were solubilized in either phosphate buffer or DMSO (nomifensine), and were injected by free-hand technique into the right striatum of conscious mice, under a volume of 1 µl. This was done according to Bizière et al, 1985. The number of complete turns was counted between 2 and 4, 8 and 10, 13 and 15 min after injection.

The vehicle-injected mice exhibited a weak short-lasting ipsilateral turning (+ 0.7 ± 0.3 to + 4.1 ± 0.4 turns/6 min). Apomorphine (Ap) induced a dose-dependent contralateral turning (mean ± SEM nb. of turns/6 min - 0.005 µg : - 1.8 ± 0.8 ; 0.01 µg : - 5.3 ± 1.1 ; 0.05 µg : - 11.9 ± 1.7 ; 0.1 µg : - 21.6 ± 1.9 ; 1 µg : - 10.5 ± 2.0) which is followed by behavioural activation. D-amphetamine (Am) induced the same effect (1 µg : - 2.6 ± 0.7 ; 10 µg : - 4.6 ± 1.8 ; 30 µg : - 13.6 ± 2.8), as did nomifensine (0.1 µg : - 1.8 ± 0.6 ; 1 µg : - 7.0 ± 1.4 ; 10 µg : - 9.9 ± 1.3 ; 30 µg : - 17.8 ± 1.5) and the atypical DAergic drug, minaprine (Bizière et al, 1984 ; 0.01 µg : - 5.5 ± 0.8 ; 0.1 µg : - 14.1 ± 1.6 ; 1 µg : - 16.3 ± 1.4). The specific $D_1$ agonist SKF 38393 also induced contralateral rotations (0.1 µg : - 5.5 ± 0.8 ; 0.5 µg : - 10.0 ± 1.1 ; 1 µg : - 13.5 ± 1.4).

The neuroleptic haloperidol (H 0.001-1 µg) and the selective $D_1$ antagonist SCH 23390 (0.1-20 µg) did not induce rotations. The turning effect of Ap was antagonized by i.p. haloperidol (H) (Ap 0.5 µg : - 13.5 ± 1.5 ; Ap + H 0.1 mg/kg : - 2.4 ± 0.6**), by s.c. SCH 23390 (Ap 0.5 µg : - 12.2 ± 1.2 ; Ap + SCH 0.001 mg/kg : - 10.5 ± 1.8 ; Ap + SCH 0.01 mg/kg : - 5.3 ± 1.3** ; Ap + SCH 0.1 mg/kg : - 1.6 ± 0.3**), but not by i.p. α-MpT (Ap 0.5 µg : - 11.7 ± 0.9 ; Ap + α-MpT 250 mg/kg : - 13.1 ± 1.6). The activity of Am was blocked by both H and α-MpT (Am 30 µg : - 16.4 ± 1.7 ; Am + H 0.1 mg/kg : - 3.6 ± 0.5** ; Am + α-MpT 250 mg/kg : - 1.8 ± 0.4**). Finally, SKF 38393-induced rotations were dose-dependently antagonized by SCH 23390 (SKF 1 µg : - 14.2 ± 1.8 ; SKF + SCH 0.001 mg/kg : - 9.2 ± 1.2* ; SKF + SCH 0.01 mg/kg : - 5.2 ± 1.0** ; SKF + SCH 0.1 mg/kg : - 0.5 ± 0.2**).

These data indicate that (i) DAmimetic drugs, injected directly into the mouse striatum, induce a contralateral turning, thus confirming previous results obtained in the rat only (McKenzie et al, 1972 ; cf. Pycock, 1980) ; (ii) the direct agonist Ap appears much more potent than the indirect DAmimetics Am or N ; (iii) this model allows a "classical" pharmacological analysis of DA-mediated mechanisms since the effect of Ap is blocked by neuroleptics but not by α-MpT, whereas the effect of Am is antagonized by both types of drugs ; (iv) a $D_1$ agonist SKF 38393 induces the same qualificative effect as the mixed agonist Ap. In conclusion, we propose this model as a useful screening tool for DAmimetic agents, since it is very simple to handle and rapid.

* $p < 0.05$ ; ** $p < 0.01$ vs intrastriatal drug alone (Student's t test)

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# HIGH DOSES OF INDANIDINE RELEASE ADRENALINE FROM THE RAT ADRENAL GLANDS

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Indanidine (Sgd 101/75) increases BP in the rat by stimulating vascular $\alpha_1$ - adrenoceptors (Weetman etal, 1983; Mathy etal, 1984), but there are marked differences in the cardiovascular effects in pithed and urethane anaesthetised animals. In pithed rats the dose-DBP response curve for indanidine was sigmoidal, and high does (4-120 µmol/kg) caused tachycardia (unpublished results). When rats were anaesthetised with urethane, the rise in DBP consisted of two distinct regressions separated by a plateau between 0.5 - 22 µmol/kg, where increasing the dose of indanidine failed to augment the increase in DBP (Chadwick et al 1983). There was no tachycardia to indanidine in these rats (up to 80 µmol/kg).

Female Sprague Dawley rats (200-250 g) were either anaesthetised with ether and then pithed, or anaesthetised with urethane (1.5 g/kg). In each rat, the trachea, carotid artery and jugular vein were cannulated so that DBP and HR could be recorded on Devices MX2 or Grass 79D recorders. In some rats, the blood vessels associated with the adrenal glands were ligated (hereafter described as adrenalectomised). Surgery was followed by a 30 min stabilisation period. Noradrenaline (NA, 1.8-59 n mol/kg) was administered i.v. at 5 min intervals in an ascending series of doses, followed by a single dose of indanidine (0.04-120 µmol/kg).

In pithed rats, the dose-vasopressor curve for indanidine was unchanged by 30 min pretreatment with propranolol (4 µmol/kg) or adrenalectomy. Indanidine-induced tachycardia was reduced in these experiments (mean values and fiducial limits, P = 0.05 : e.g. indanidine 120 µmol/kg, control tachycardia 108 (61-156), n = 5; after propranolol + 20 (10-30), n = 4 : after adrenalectomy +3 (-6 — + 12) n = 4).

In urethane-anaesthetised rats, the rise in DBP to indanidine (1.6 - 50 µmol/kg) was greater in adrenalectomised rats than in controls, this effect being most prominent with high doses (> 1 µmol/kg). The triphasic nature of the dose-response curve for indanidine in control rats was not found in those that had been adrenalectomised; instead, it was sigmoidal, much like that in pithed rats. Propranolol (4µmol/kg) also rectified the dose-DBP curve in anaesthetised rats.

It is concluded that in rats anaesthetised with urethane, high doses of indanidine (1 µmol/kg and above) release a vasodilator substance from the adrenal glands (probably adrenaline) which opposes the rise in DBP due to the vasoconstrictor action of indanidine on vascular $\alpha_1$ - adrenoceptors, thus causing the plateau in the dose-response curve.

We would like to thank Dr. A. G. Roach for advice and Siegfried AG, Zofingen, Switzerland for financial support.

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## 387P

# EFFECT OF DEXAMETHASONE ON 7-ALKOXYCOUMARIN O-DEALKYLASE ACTIVITY OF RAT HEPATOCYTES IN PRIMARY CULTURE

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We and others have reported that the inclusion of dexamethasone (D) into the medium used to culture rat hepatocytes attenuated the loss of cytochrome P450-dependent 7-ethoxycoumarin O-deethylase (7-ECOD) activity that occurred during the first 24 hours of placing such cells in culture (Edwards et al 1984; Warren et al 1985). We have previously demonstrated that the O-dealkylations of 7-methoxycoumarin (7-MCOD) and 7-ethoxycoumarin are mediated by different forms of cytochrome P450 (Paterson et al 1984) and we have now compared the effect of D on 7-MCOD and 7-ECOD activities of rat hepatocytes in primary culture.

Isolation and culture of hepatocytes from male rats were carried out as described previously, as were the measurement of 7-ECOD activity and DNA content (Warren et al 1985). 7-MCOD activity was measured by the method used for 7-ECOD. The culture medium was Eagle's M.E.M. with 10% foetal calf serum and D (1 μM) was added on initiation of the culture. Statistical analysis used a paired sample Student t-test. The results are presented in Table 1 (values are mean±s.e. mean of 6 separate experiments).

Table 1. Effect of D on 7-MCOD and 7-ECOD activities of rat hepatocytes in primary culture (activities expressed as pmol product/μg DNA/4 h)

| Activity Measured | Culture Conditions | Time in culture (h) | | |
|---|---|---|---|---|
| | | 0 | 24 | 72 |
| 7-MCOD | -D | 515±21 | 217±10 | 45± 6 |
| | +D | | 319± 8[+] | 119± 6[+] |
| 7-ECOD | -D | 945±57 | 461±32 | 228±11 |
| | +D | | 807±21[+] | 1715±167[+] |

[+]Significantly different from control (-D) at $P<0.001$.

There was a decrease of approx. 50% in the activity of both enzymes during the first 24 h of culture in standard medium, and the presence of D attenuated these decreases. After 72 h 7-ECOD activity was further reduced in cells cultured in standard medium, but was significantly increased in the presence of D when compared to that in freshly-isolated cells ($P<0.05$) or that in cells cultured for 24 h ($P<0.01$); this was probably due to enzyme induction (Edwards et al 1984). 7-MCOD activity also decreased further in cells cultured in standard medium at 72 h. 7-MCOD activity in cells cultured in the presence of D, whilst higher than that in cells cultured in the absence of D, was significantly ($P<0.001$) less than that measured in freshly-isolated cells or cells cultured for 24 h. This inductive effect of D on 7-ECOD activity was not related to induction of a form of cytochrome P450 inducible by pregnenolone-16α-carbonitrile (PCN) as proposed by Schuetz et al (1984), since PCN itself (1-100 μM) did not induce 7-ECOD activity.

It is suggested that D exerts a maintenance effect on cytochrome P450-related enzyme activity during the first 24 h in culture, and thereafter exerts a selective inductive effect on certain of the multiple forms of cytochrome P450.

The financial assistance of FRAME is gratefully acknowledged.

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