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FOREWORD

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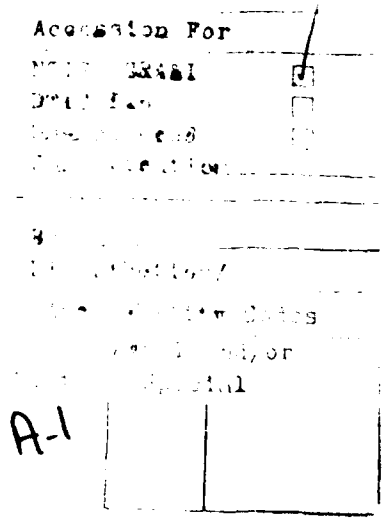

A.

EVALUATION OF DEXTROMETHORPHAN AND ITS ANALOGUES IN DEXTROMETHORPHAN (^{3}H-DM), THIENYLCYCLOHEXYLPIPERIDINE (^{3}H-TCP) AND GLYCINE (^{3}H-GLYCINE) BINDING ASSAYS

METHODS

Binding of dextromethorphan and its analogues to the dextromethorphan binding site and to the PCP and glycine binding sites associated with the N-methyl-D-aspartate receptor-channel complex was investigated using ^{3}H-DM, ^{3}H-TCP and ^{3}H-glycine as radioligands. The methods used were based on those reported in the literature (as given below), but have been optimised for the conditions described. The experiments were carried out in 1ml tubes (miniblocks), in a final incubation volume of 0.5ml. All reagents were diluted and dispensed using a TECAN and filtering was done using a Brandel M-48R. Samples were counted in 5ml of Esoscint A. after an extraction period of at least 6 hours, using a Beckman LS1701 liquid scintillation counter (with automatic corrections for quenching and efficiency). All compounds were dissolved in distilled water except compound 3 which was dissolved in DMSO/lactic acid and diluted with distilled water (final concentration each solvent <0.01% @ 10^{-5}M). Standards were obtained from commercial sources except dichlorokynurenic acid (DCK) which was a gift from Pfizer Central Research, Sandwich, Kent.

1. ^{3}H-Dextromethorphan (^{3}H-DM) Binding

Tissue Prep. Frozen brains (male CD rats or DH guinea-pigs, Charles River) were thawed, suspended in 10 volumes of ice-cold 0.32M sucrose and homogenised using a Polytron (30 seconds, setting 6). The suspension was then centrifuged at 1000g for 20 minutes and the pellet (P_1) discarded. For the rat brain tissue preparation, the supernatant from the 1000g spin was centrifuged at 100,000g for 60 minutes, and the pellet resuspended in 2.5 volumes of 50mM Tris-HCl (pH 7.4 @ 25°C) and frozen at -80°C. Guinea-pig brain sub-cellular fractions were prepared based on the method of De Robertis *et al.* (1962). The supernatant from the 1000g spin was first centrifuged at 40,000g for 20 minutes The pellet was resuspended in 10 volumes of ice-cold Tris-HCl, and recentrifuged at 11,500g for 20 minutes. This pellet (crude mitochondrial, P_2) was washed once in Tris-HCl, resuspended in 2.5 volumes of Tris-HCl (as for rat brain) and frozen at -80°C. The supernatant from the 20,000g spin was centrifuged at 100,000g for 60 minutes to give the microsomal pellet (P_3). This was resuspended in 2 volumes of Tris-HCl (as above) and frozen at -80°C.

^{3}H-DM Binding Assay: The method was based on one developed by Craviso and Musacchio (1983). 50µl of ^{3}H-DM (NEN, specific activity 85.3Ci/mmol) at a final concentration of 5nM, were incubated for 20 minutes at 23°C with 400µl of either rat or guinea-pig brain membranes (approximately 0.3mg rat brain and g. inea-pig P_2 and 0.5mg guinea-pig P_3), and 50µl of buffer (to define total binding), cold dextromethorphan at 100µM (to define non-specific binding) or varying concentrations of displacing compound (10^{-9} to 10^{-5}M). The reaction was terminated by dilution with ice-cold wash buffer (50mM Tris-HCl, pH 7.4 @ 0°C, containing 100mM choline chloride and 0.01% Triton X-100), followed by rapid filtration through GF/B filters presoaked >2 hours in wash buffer (to reduce non-specific binding of ^{3}H-DM to the filters). The filters were washed 4 times with 1ml wash buffer, and counted as described above.

2. ^{3}H-TCP and ^{3}H-Glycine Binding

Tissue Prep. The method was based on that developed by Bristow *et al.* (1986). Frozen rat brains were thawed and suspended (minus cerebellum) in 10 volumes of ice-cold 0.32M sucrose and homogenised using a Polytron (30 seconds, setting 6). The homogenate was then centrifuges at 1000g for 20 minutes, the pellet discarded and the supernatant centrifuged at 20,000g for 20 minutes. The pellet from this resuspended in 10 volumes of ice-cold distilled water, vortexed and allowed to stand for 10 minutes before being centrifuged at 8,000g for 20 minutes. The supernatant was then used to wash the buffy uppercoat away from the brown mitochondria-enriched pellet, and centrifuged at 40,000g for 20 minutes to give crude synaptic plasma membranes. These were washed twice in distilled water and the pellets frozen at -20°C for at least 18 hours. They were then thawed and resuspended in 10 volumes of either 5mM Tris-HCl (pH 7.4 @ 0°C) for the TCP assay, or 50mM Tris-acetate (pH 7.7 @ 0°C) for the glycine assay, vortexed and washed a further 6 times by centrifuging at 40,000g with a 10 minute incubation at room temperature between each spin. The final pellet was resuspended in 2.5 volumes of either TCP buffer or glycine buffer, and frozen at -80°C. On the day of the experiment, the tissue was thawed and washed once more in 4 volumes of the appropriate buffer before use in the assay.

^{3}H-TCP Binding Assay: The method was a modification of one developed by Monahan *et al.* (1989). 50µl of ^{3}H-TCP (NEN, specific activity 40.8Ci/mmol) at a final concentration of 5nM, were incubated for 60 minutes at 23°C with 400µl of rat brain crude synaptic plasma membranes prepared as described above (approximately 0.2mg) and 50µl of TCP buffer (for total binding), cold phencyclidine (PCP, for non-specific binding), or varying concentrations of displacing compound (10^{-9} to 10^{-5}M). The reaction was terminated by dilution with ice-cold wash buffer (5mM Tris-HCl, pH 7.7 @ 0°C), followed by filtration through GF/B filters presoaked for at least 2 hours in 5mM Tris-HCl containing 0.5% polyethyleneimine (PEI) to reduce filter binding. The filters were then washed 4 times with 1ml of wash buffer, and counted as given previously.

^{3}H-Glycine Binding Assay: This method was modified from those employed by Bristow *et al.* (1986) and Sacaan *et al.* (1989). 50µl of ^{3}H-glycine (NEN. specific activity 43Ci/mmol), at a final concentration of 25nM, were incubated for 15 minutes at 0°C with 400µl of rat brain crude synaptic plasma membranes, and 50µl of either glycine buffer (total binding), 1mM D-serine (non-specific binding) or varying concentrations of displacing compound (10^{-8} to 10^{-3}M, except glycine which was tested from 10^{N}M to 10^{-4}M). The reaction was terminated by dilution with ice-cold was buffer (50mM Tris HCl, pH 7.7 @ 0°C containing 10mM

$MgCl_2$, to reduce dissociation of the ligand receptor complex during the separation procedure), followed by rapid filtration through GF/B filters presoaked in Tris-acetate containing 0.1% PEI and 1mM glycine (to reduce filter binding). The filters were then washed 3 times with 1ml of wash buffer, and the filters counted as before. The duration of the separation procedure was less than 6 seconds, and the reaction mixture and reagents was kept below 4°C, to minimise dissociation of the fairly low affinity ^{3}H-glycine from its binding site.

RESULTS

The results are shown in Table 1. Values given are expressed in micromolar where a displacement curve was obtained, or in percentage inhibition at 10^{-5}M where this was less than 50%, or where an abnormally high slope was obtained. In addition to the analogues of dextromethorphan, standards for each assay were also tested (dextromethorphan for ^{3}-DM binding, phencyclidine (PCP) for ^{3}H-TCP binding, and glycine for ^{3}H-glycine binding), as well as chlorpromazine (CPZ), MK-801 and dichlorokynurenic acid (DCK) in all 5 assays.

None of the compounds tested were able to displace ^{3}H-glycine; in fact in most cases an apparent stimulation of binding was observed. In addition, DCK, a ligand reported to be more potent than glycine at the glycine-binding site, was inactive against ^{3}H-DM, while being active against both ^{3}H-TCP and ^{3}H-glycine. All the compounds except 1 were less active against ^{3}H-TCP than against ^{3}H-DM; this compound was about 2-fold more potent than dextromethorphan against ^{3}H-TCP. In general, the compounds were more active in guinea-pig than in rat brain, although this may reflect the fact that guinea-pig was tested as P_2 and P_3 fractions, and does not rule out the possibility that a compound may be active in either of these fractions in the rat brain. Dextromethorphan is reported to be more active in the microsomal fraction of guinea-pig brain, although this was not reflected in the results obtained in these experiments. The reason for this is not clear, but may reflect the fact that whole brain was used (high affinity ^{3}H-DM binding is enriched in specific brain regions especially pons-medulla and cerebellum). It could be due to contamination of the preparation by other cell fractions, although this seems unlikely in view of the initial 40,000g spin employed to isolate this fraction. It is unlikely that dextromethorphan is binding to the same site as ^{3}H-TCP as MK-801 showed little activity against ^{3}H-DM binding as did DCK. Several of the compounds appeared to be more potent than dextromethorphan in the ^{3}H-DM binding assays, notably 48 in rat brain; 46,47 and 48 in guinea-pig P_2; and 7,8,32,46,47 and 48 in guinea-pig P_3. Only compound 3 showed little activity against any of the ligands tested. More detailed conclusions may be drawn based on the structures of the compounds relative to dextromethorphan.

Some of these data have been presented to the British Pharmacological Society Meeting (July 1992, Dublin), abstract attached. A series of carbapentane analogues (synthesised by Amy Newman) are currently being evaluated in binding assays for ^{3}-DM in rat and guinea-pig brain and ^{3}H-TCP in rat brain. This should be completed soon.

REFERENCES

1. De Robertis *et al.* (1989) **J. Neurochem.** 9:23-35.
2. Musacchio *et al.* (1989) **Mol. Pharmacol.** 35:1-5
3. Bristow *et al.* (1986) **Eur. J. Pharmacol.** 126:303-7
4. Monahan *et al.* (1989) **J. Neurochem.** 53: 370-5
5. Sacaan & Johnson (1989) **Mol. Pharmacol.** 36:836-9
6. Craviso & Musaccio (1983) **Mol. Pharmacol.** 23:618-28
7. Baron *et al.* (1991) **Eur. J. Pharmacol.** 206: 149-54

TABLE 1

COMPOUND	IC50 (micromolar)									
	3H-DM						3H-TCP		3H-GLYCINE	
	G.Pig P2		G.Pig P3		rat brain		rat SPM		rat SPM	
DM	0.24+/-0.06	(8)	0.49+/-0.12	(9)	0.59+/-0.12	(10)	2.0+/-0.6	(4)	N/A	(4)
1	2.5+/-0.4	(4)	2.7+/-0.3	(5)	2.5+/-0.9	(5)	1.2+/-0.7	(4)	N/A	(4)
3	25% @ 10-5M	(4)	50% @ 10-5M	(4)	19% @ 10-5M	(5)	N/A	(4)	N/A	(6)
4	6.4+/-2.8	(4)	4.4+/-0.9	(5)	45% @ 10-5M	(5)	7.8+/-1.4	(3)	N/A	(4)
5	0.48+/-0.12	(4)	1.3+/-0.7	(4)	3.6+/-1.4	(5)	43% @ 10-5M	(4)	N/A	(4)
6	0.68+/-0.13	(4)	1.1+/-0.5	(5)	4.4+/-0.9	(5)	45% @ 10-5M	(4)	N/A	(4)
7	0.37+/-0.12	(4)	0.21+/-0.03	(4)	1.1+/-0.4	(5)	5.5+/-1.5	(4)	N/A	(6)
8	0.44+/-0.13	(4)	0.17+/-0.01	(4)	1.5+/-0.3	(5)	60% @ 10-5M	(4)	N/A	(6)
10	1.7+/-0.3	(4)	1.0+/-0.3	(5)	1.3+/-0.3	(5)	53% @ 10-5M	(4)	N/A	(4)
32	0.27+/-0.07	(4)	0.15+/-0.07	(4)	3.1+/-0.6	(5)	39% @ 10-5M	(4)	N/A	(6)
46	0.16+/-0.03	(4)	0.10+/-0.04	(4)	1.5+/-0.4	(5)	58% @ 10-5M	(4)	N/A	(5)
47	0.15+/-0.05	(4)	0.12+/-0.03	(4)	0.88+/-0.18	(5)	59% @ 10-5M	(4)	N/A	(5)
48	0.09+/-0.02	(4)	0.13+/-0.03	(4)	0.42+/-0.06	(5)	75% @ 10-5M	(4)	N/A	(5)
CPZ	1.1+/-0.10	(3)	0.60+/-0.15	(4)	1.3+/-0.2	(3)	25% @ 10-5M	(4)	N/A	(4)
MK-801	19% @ 10-5M	(4)	20% @ 10-5M	(4)	42% @ 10-5M	(3)	0.01+/-0.003	(4)	10% @ 10-5M	(4)
DCK	N/A	(4)	N/A	(3)	N/A	(2)	2.4+/-1.3	(4)	0.39+/-0.35	(2)
PCP	-		-		-		0.18+/-0.04	(7)	-	
glycine	-		-		-		-		0.69+/-0.27	(9)

DCK = dichlorokynurenic acid
CPZ = chlorpromazine

( ) = n value
N/A = no effect

SPM = synaptic plasma membranes

Mechanism of the anticonvulsant action of dextromethorphan analogues in rat brain

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Dextromethorphan (DM), a non-opioid cough suppresant, has recently been discovered to possess anticonvulsant and neuroprotective properties (Tortella *et al.*, 1989); however, its mechanism of action has not yet been elucidated. DM binds to high affinity sites in guinea-pig and rat brain, and with lower affinity to the NMDA receptor-associated phencyclidine (PCP) site. DM is rapidly metabolised to dextrorphan (DX), which could mediate the anti-convulsant and neuroprotective properties of DM *in vivo*.

To investigate further the mechanism of the anticonvulsant action of DM, a series of (+)-3-substituted-17-methylmorphinans have been synthesised (Table 1). These compounds are either not expected to be metabolised, or to do so at a reduced rate as compared to DM. Anticonvulsant potency of the DM analogues was assessed using the rat supramaximal electroshock (MES) model (Tortella *et al.*, 1986) and compared with the binding profile at the DM and PCP sites in rat brain membranes, using [^{3}H]DM (Craviso *et al.*, 1983), and [^{3}H]thienylcyclohexylpiperidine ([^{3}H]TCP; Monahan *et al.*, 1989) respectively.

DM, DX and compounds, 5, 14 and 15 possessed potent anticonvulsant activity with ED_{50} values in $\mu molkg^{-1}$ (95% confidence limits), of 109 (54-211), 12 (3-69), 76 (49-116), 17.4 (9-33.2) and 9.4 (5.5-15.9) respectively, and providing 70-100% protection. Compounds 7, 16 and 17 were less potent and gave only partial protection (30-40%) against seizures at the highest doses used (40-50$mgkg^{-1}$). Compounds 3, 6, 8, 9 and 11 were inactive at these doses. All of the analogues with the exception of 3 and 5 (>10μM) bound with appreciable affinity to the [^{3}H]DM site in rat brain membranes, with 14 (0.42 ± 0.06μM) and 15 (0.88 ± 0.18μM) being equipotent with DM (0.59 ± 0.12μM), and the other compounds ranging from 1-4μM. For compounds with demonstrable *in vivo* activity, there was a good correlation between anticonvulsant activity and potency at the DM binding site in rat brain. The lack of anticonvulsant activity of 6, 8, 9 and 11 may be due to lack of brain penetration or differences in DM receptor sub-types. With the exception of DX, 5 and 8, all the compounds were more than 10-fold less potent at the PCP site than the DM site. It is concluded that binding to the DM site may mediate the anticonvulsant activity of the DM analogues 7, 14, 15, 16 and 17 and that metabolism to DX may not necessarily be required for DM to produce its effects *in vivo*.

Table 1: 3-substituted-17-methylmorphinans

DM - OCH_3	8 - Cl
DX - OH	9 - NCS
3 - 2-phenyl-4-quinazolinyloxy	11 - H
5 - NH_2	14 - OCH_2CH_3
6 - $NHCH_3$	15 - $OCH(CH_3)_2$
7 - $N(CH_3)_2$	16 - $OCH_2CH_2CH_2CH_3$
	17 - OCH_2Ph

Tortella F.C., Pellicano M., and Bowery N.G. (1989) Trends Pharmacol. Sci. **10** 505-507

Tortella F.C., Robles L., Holaday J.W. (1986) J. Pharmacol. Exp. Ther. **237** 49-53

Craviso G.L., Musacchio J.M. (1983) Mol. Pharmacol. **23** 619-628

Monahan J.B., Corpus V.M., Hood F.W., Thomas J.W., Compton R.P. (1989) J. Neurochem. **53** 370-375

B.

STUDIES ON DEXTROMETHORPHAN AND ANALOGUES IN A RAT MODEL OF CEREBRAL ISCHAEMIA

The rat middle cerebral artery occlusion technique (MCA) has been established during this period. The first objective was to ensure that the ischaemic infarct obtained with each MCA was of a reproducible size consistent with data already quoted in the literature. This was achieved and the results obtained are summarized in Table 1. The infarct size was measured in 400μm transverse sections cut sequentially through the brain. Sections were stained with tetrazolium chloride (TTC) and the damaged area measured from photographic images prepared for each section at the same magnification. Each area was defined by image analysis using a Quantimet 970 analyser. All sections were prepared 3 days after the arterial occlusion.

Having established the occlusion technique, the binding of ^{3}H-dextromethorphan (^{3}H-DM) to different brain regions will be examined to determine at what time and whether changes occur within and outside the infarct area. This will be studied using receptor autoradiography as well as homogenates of brain regions.

As an initial control step it has been necessary to ensure that freezing does not affect the binding of ^{3}H-DM differentially between normal and infarcted brain tissue. Crude membrane homogenates were prepared from fresh or frozen brain tissue from lesioned rats by homogenisation in 50mM TRIS-HCl, pH 7.4 at 23°C and the 1000g pellet (P_1 nuclear fraction) discarded. The remaining suspension which was used for the binding assay contained the P_2 and P_3 pellets (mitochondrial and microsomal fractions) and also the S_3 supernatant (cytosol). Full displacement curves for unlabelled dextromethorphan against ^{3}H-DM binding were then obtained in both normal and ischaemic brain comparing both sides of each brain separately. The results obtained are summarized in Table 2. On comparing the groups by analysis of variance no significant differences between fresh and frozen brain on the ipsilateral or contralateral sides was observed.

The next stage of the study will be to examine the binding of ^{3}H-DM in a more quantitative manner using saturation analysis. This will allow the determination of the affinity constant (K_d) and specific binding capacity (B_{max}) of ^{3}H-DM in both normal (sham-operated) and ischaemic (MCA-O) rat brain. Again, both ipsilateral and contralateral sides of each brain will be tested, and each brain will be tested individually (rather than pooled for each group). For comparison, the binding characteristics of two other ligands will also be investigated. PK-11195,

a peripheral benzodiazepine ligand, has been shown to be an accurate and reliable marker of neuronal damage. This ligand appears to bind to glial and other non-neuronal cells which proliferate during ischaemic degeneration. Binding of PK-11195 increases markedly after middle cerebral artery occlusion, first becoming detectable at 2 days post occlusion and reaching a maximum 8 days post occlusion. The other ligand to be investigated will be ^{3}H-DTG (^{3}H-N$_{1}$N-di(0-tolyl)guanidine), a ligand which binds with relative selectivity to the sigma receptor, but which does not discriminate between sigma$_{1}$ and sigma$_{2}$ receptors. As ^{3}H-DM binds to sigma$_{1}$ but not sigma$_{2}$ receptors, this ligand could indicate whether the sigma receptor is involved in the neuroprotective effect of DM. To date, all of the MCA-O rats required for this study have been prepared, and are awaiting binding experiments.

Once this series of experiments is complete, the autoradiographic distribution of ^{3}H-DM in normal and ischaemic rat brain will be determined. This will be particularly interesting if previous results indicate that there are differences in ^{3}H-DM binding characteristics in the ischaemic brain.

Table 1. Infarct size in MCA-O rat brain

SECTION	INFARCT SIZE (mm^2) ± S.E.M.
1 (front)	10.4 ± 0.9
2	14.5 ± 0.6
3	14.3 ± 0.9
4	12.1 ± 0.7
5 (back)	8.2 ± 0.9

Table 2. A comparison of fresh and frozen rat brain.

Tissue	Dextromethorphan IC_{50} & slope (nM ± S.E.M.)	
	Fresh	Frozen
Ipsilateral	157.5 ± 16.1 -0.69 ± 0.06	147.5 ± 23.0 -0.68 ± 0.05
Contralateral	359.1 ± 93.2 -0.73 ± 0.13	222.1 ± 54.1 -0.69 ± 0.04