Pharmacology Biochemistry & Behavior, Vol. 24, pp. 23–25, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

2-Methyl-3,3-Diphenyl-3-Propanolamine (2-MDP) Selectively Antagonises N-Methyl-Aspartate (NMA)

J. C. BLAKE, S. N. DAVIES, J. CHURCH, D. MARTIN AND D. LODGE[1]

Department of Physiology, Royal Veterinary College, Royal College Street, London, NW1 OTU

Received 25 February 1985

BLAKE, J. C., S. N. DAVIES, J. CHURCH, D. MARTIN AND D. LODGE. *2-Methyl-3,3-diphenyl-3-propanolamine (2-MDP) selectively antagonises N-methyl-aspartate (NMA).* PHARMACOL BIOCHEM BEHAV **24**(1) 23–25, 1986.—Using electrophoretic application to rat central neurones in vivo, and bath application to frog spinal cord in vitro, 2-methyl-3,3-diphenyl-3-propanolamine was found to be a selective antagonist of N-methyl-DL-asparate, but not of quisqualate or kainate. In this respect the (−) isomer proved to be about three times more potent than the (+) in both preparations.

2-Methyl-3,3-diphenyl-3-propanolamine Excitatory amino acids Phencyclidine

SEVERAL arylcyclohexylamines, e.g., phencyclidine (PCP), and some dioxolanes, benz(f)isoquinolines, and benzomorphans of the sigma opiate type have common behavioural and neurochemical properties. In particular they all produce a form of cataleptic anaesthesia, have psychomimetic properties in man, cannot be easily separated in drug discrimination studies and bind at a common site in CNS tissue, namely the PCP/sigma opiate receptor site ([4, 5, 6, 8, 12] and see [1, 2, 7]). Since these substances also selectively antagonise the glutamate/aspartate analogue, N-methyl-aspartate (NMA) [1, 2, 7], we have proposed that block of central synapses utilizing NMA receptors may underlie some of the behavioural properties common to these groups of compounds [7]. The recent report by Tang, Cangelosi, Code and Franklin [9] demonstrating that 2-MDP, in particular the (−) isomer, has anaesthetic and behavioural properties common to PCP, gave us the opportunity to test the above hypothesis further by investigating whether this substance was also a selective NMA antagonist. We have, therefore, tested the effects of (+) and (−) 2-MDP on responses of rat neurones in vivo, and frog neurones in vitro, to the administration of NMA, quisqualate and kainate, the three ligands used for characterising central glutamate/aspartate receptors [11].

METHOD

The methods used for electrophoretic experiments have been described in detail elsewhere [1]. Briefly, rats were anaesthetised with sodium pentobarbitone (50 mg/kg IP and supplemented IV as necessary) and either the lumbar spinal cord or the medulla exposed. Extracellular recordings were made with seven barrel micropipettes from unidentified single units. The centre barrel containing 3.6 M NaCl was used for recording extracellular action potentials of single neurones, the firing rate of which was displayed on a chart recorder. Five of the six outer barrels contained a combination of the following drugs: N-methyl-DL-aspartate Na (NMA, 200 mM, pH 8.1), quisqualate Na (5 mM in 200 mM NaCl, pH 7.7), kainate Na (5 mM in 200 mM NaCl, pH 8.2), the (+) and (−) isomers of 2-methyl-3,3-diphenyl-3-propanolamine HCL (2-MDP, 25 mM in 175 mM NaCl, pH 4.5), (±) ketamine HCl (25 mM in 175 mM NaCl, pH 4.5). The remaining outer barrel contained 200 mM NaCl and was used for current balancing. Once a cell was isolated, approximately equal and submaximal responses were obtained from the agonists before administration of (+)2-MDP, (−)2-MDP or ketamine. Results are given as mean change in response±one standard deviation.

The frog cord preparation was adapted from that of Curtis, Phillis and Watkins [3]. Briefly, the spinal cord was removed from a decapitated frog, hemisected, and stored overnight in frog Ringer at 4°C. Each half was put in a Perspex chamber and perfused with frog Ringer at 10–12°C containing 0.1 μM tetrodotoxin to abolish any synaptic activity. Amino acid agonists were added to the perfusate and the subsequent depolarisation of motoneurones was recorded from a ventral root. In each experiment a dose-response curve was obtained for one or more of the agonists, and then repeated in the presence of varying concentrations of (+) or (−) 2-MDP or ketamine.

RESULTS

No differences were noted between results from medullary or spinal cord cells and therefore the results from the

[1]Requests for reprints should be addressed to D. Lodge.

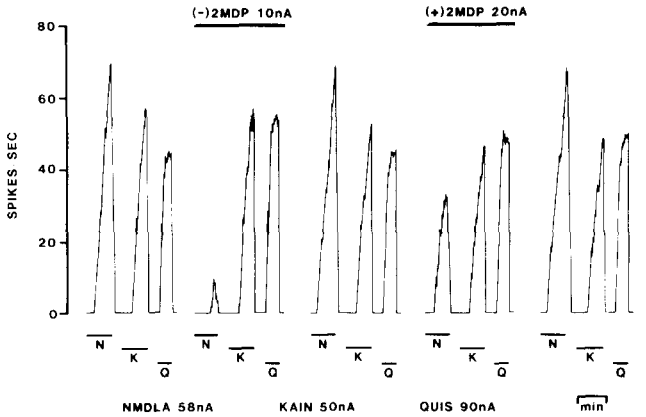

FIG. 1. The effect of the electrophoretic ejection of (−)2-MDP (10 nA) and (+)2-MDP (20 nA) upon the responses of a rat spinal neurone to NMA (58 nA), kainate (50 nA) and quisqualate (90 nA). (−)2-MDP reduced the NMA response by 86% and recovery was observed 60 minutes later. (+)2-MDP reduced the NMA response by 52% and recovery is shown 40 minutes later. Ordinate scale: firing rate in spikes/sec. Abscissa scale: time. Calibration bar=1 minute.

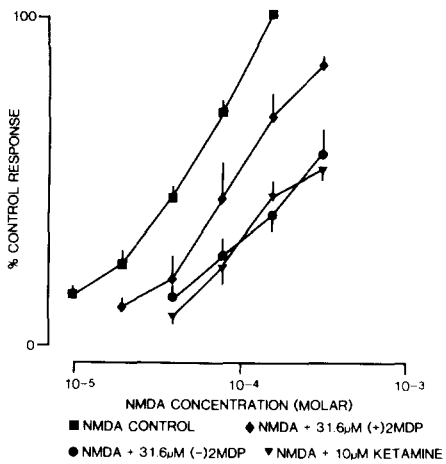

FIG. 2. Dose-response curve for motoneuronal depolarisation to the agonist NMDA alone (■), and in the presence of 31.6 μM (+)2-MDP (◆), 31.6 μM (−)2-MDP (●), and 10 μM ketamine (▼). Points shown are mean±standard error.

two sites have been pooled. Electrophoretic (−)2-MDP (mean ejection current=11.4 nA, range=5–60 nA) selectively reduced responses to NMA in all 30 cells tested (mean=67±20%), whereas responses to both quisqualate and kainate tended to increase (18±30%, N=30 and 7±13%, N=13 respectively) (see Fig. 1). At high ejection currents (−)2-MDP caused some reduction of action potential amplitude. Recovery of the NMA effect was slow, lasting up to 60 minutes, however recovery to at least 80% of control was observed in 20 of the cells, and the remaining 10 all recovered to within 50% of control. (+)2-MDP (mean ejection current=24.7 nA, range=6–50 nA) also selectively reduced NMA actions in 11 neurones tested (mean=68±30%), and in a further 6 cells spike height was reduced so as to become uncountable before any reduction in the response was observed. Responses to quisqualate and kainate were more variable, either changing little or sometimes showing dramatic increases (39±68%, N=10 and 24±41%, N=4 respectively).

With 9 cells it was possible to compare directly the effects of (−)2-MDP and ketamine on the same cell and by a comparison of ejection currents (−)2-MDP proved to be 1.5–3 times more potent an NMA antagonist than ketamine. In 5 of these (+)2-MDP was also compared and in these cases (−)2-MDP proved to be 2.5–4 times more potent than (+)2-MDP. An example of this difference in potency of the two isomers is shown in Fig. 1. On this spinal neurone the (−) isomer was 3–4 times more effective as an NMA antagonist. Note also the increase in quisqualate but not kainate responses during the ejection of the two isomers.

With 4 cells (−)2-MDP was administered IV and a dose of 4–8 mg/kg selectively reduced the NMA response by 67±8%. Recovery on these occasions was very slow and only reached 60% of control at best.

On the frog spinal cord in vitro both isomers of 2-MDP (3.16–100 μM) reduced the response to NMA, shifting the dose-response curve to the right (see Fig. 2). (−)2-MDP proved to be 3 times more potent than (+)2-MDP in this respect, the pA2 values being 5.15±0.04 and 4.64±0.05 respectively. This compares with a pA2 value for ketamine of 5.68±0.06, suggesting that ketamine is about 3 times more potent as an NMA antagonist than (−)2-MDP in this preparation. The dose-response curves to quisqualate and kainate showed no significant changes in the presence of up to 100 μM (+) or (−) 2-MDP.

DISCUSSION

We have demonstrated in both the rat in vivo, and the frog in vitro, that 2-MDP selectively antagonises responses of neurones to NMA, but not those to quisqualate and kainate. In both preparations the (−) isomer of 2-MDP proved to be about 3 times more potent than the (+) isomer. The fact that in the experiments of Tang *et al.* [9], (+) 2-MDP was devoid of PCP-like activity may be explained by the adverse behavioural side effects which prevented the testing of higher doses. Inhibition of binding of H^3-dexoxadrol, another PCP-like dissociative anaesthetic, to rat brain membranes is displaced by 2-MDP, the (−) isomer being about five times more potent than the (+) isomer (V. Sethy, personal communication). The cause of the apparent difference in potency of (−)2-MDP relative to ketamine between our two preparations may be partly a consequence of species differences and/or the different methods of administration of the agonists and antagonists.

These results extend the observed correlation between PCP-like behavioural effects and NMA antagonism to another distinct class of drugs. It is not yet clear how NMA antagonism is brought about, nor what the neuronal substrate might be for the behavioural effects of 2-MDP. Recent results, however, have shown that other NMA antagonists, e.g., ketamine, cyclazocine and aminophosphonovalerate, have profound effects on cortical synaptic transmission [10], a potential site for some of the behavioural effects common to dissociative anaesthetics and sigma opiates.

ACKNOWLEDGEMENTS

We would like to thank Dr. Shinozaki, Warner-Lambert UK Ltd., and Dr. Tang of Upjohn Ltd., for kind gifts of quisqualate, ketamine and 2-MDP respectively. This work was supported by a grant from the MRC. J.C.B. was a Wellcome vacation scholar. J.C. is an MRC training fellow.

REFERENCES

1. Anis, N. A., S. C. Berry, N. R. Burton and D. Lodge. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br J Pharmacol* **79:** 565–575, 1983.
2. Berry, S. C. and D. Lodge. Benz(f)isoquinolines as excitatory amino acid antagonists: an indication of their method of action? *Biochem Pharmacol* **33:** 3829–3832, 1984.
3. Curtis, D. R., J. W. Phillis and J. C. Watkins. Actions of amino acids on the isolated hemisected spinal cord of the toad. *Br J Pharmacol* **16:** 262–283, 1961.
4. Domino, E. F. Neurobiology of phencyclidine (Sernyl), a drug with an unusual spectrum of pharmacological activity. *Int Rev Neurobiol* **6:** 303–347, 1964.
5. Herling, S. and J. H. Woods. Discriminative stimulus effects of narcotics: evidence for multiple receptor-mediated actions. *Life Sci* **28:** 1571–1587, 1981.
6. Kamenka, J.-M., E. F. Domino and P. Geneste. *Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications*. Ann Arbor: NPP Books, 1983.
7. Lodge, D. and S. C. Berry. Psychotomimetic effects of sigma opiates may be mediated by block of central excitatory synapses utilising receptors for aspartate-like amino acids. In: *Modulation of Sensorimotor Activity During Alterations in Behavioural States*, edited by R. Bandler. New York: Alan R. Liss, 1984, pp. 503–518.
8. Shannon, H. E. Pharmacological evaluation of N-allylnormetazocine (SKF 10,047) on the basis of its discriminative stimulus properties in the rat. *J Pharmacol Exp Ther* **225:** 144–152, 1983.
9. Tang, A. H., A. A. Cangelosi, R. A. Code and S. R. Franklin. Phencyclidine-like behavioural effects of 2-methyl-3,3-diphenyl-3-propanolamine (2-MDP). *Pharmacol Biochem Behav* **20:** 209–213, 1984.
10. Thompson, A. M., D. C. West and D. Lodge. An N-methylasparate receptor-mediated synapse in rat cerebral cortex: a site of action of ketamine? *Nature* **313:** 479–481, 1985.
11. Watkins, J. C. and R. H. Evans. Excitatory amino acid transmitters. *Annu Rev Pharmacol Toxicol* **21:** 165–204, 1981.
12. Zukin, R. S. and S. R. Zukin. Demonstration of (3H)-cyclazocine binding to multiple opiate receptor sites. *Mol Pharmacol* **20:** 246–254, 1981.