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BENZ(*F*)ISOQUINOLINES AS EXCITATORY AMINO ACID ANTAGONISTS: AN INDICATION OF THEIR MECHANISM OF ACTION?

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Abstract—Using the technique of microelectrophoresis on cat and rat spinal neurones, the bridged benz(*f*)isoquinoline, LY154045, like ketamine and dextrorphan, was found to be a selective antagonist of *N*-methylaspartate, an amino acid used for characterizing excitatory amino acid synaptic receptors. The unbridged analogue, LY154005, was inactive as an amino acid antagonist. This result correlates well with the ability of LY154045, but not LY154005, to displace phencyclidine from CNS tissue and to mimic phencyclidine in behavioural tests. The potential role of *N*-methylaspartate antagonism in the aetiology of some of the behavioural effects of LY154045, phencyclidine and related drugs is considered.

A novel group of pharmacological agents, based on benz(*f*)isoquinoline structures, has recently been shown to have both behavioural and neurochemical properties in common with phencyclidine and sigma opioids [1, 2]. The potencies of these compounds relative to dissociative anaesthetics and psychotomimetic benzomorphans as inhibitors of phencyclidine binding to CNS tissue correlated well with their potencies in drug discrimination studies.

We have recently provided evidence that some of the neuropharmacological effects of these latter two groups of compounds may result from reduced synaptic excitation mediated via receptors for excitatory amino acids. Thus dissociative anaesthetics, such as phencyclidine [3], ketamine [3] and etoxadrol [4] and benzomorphans such as cyclazocine [5] and *N*-allylnormetazocine [6], are all selective antagonists of *N*-methylaspartate (NMA), a ligand used for classifying one of the three recognized classes of excitatory amino acid receptor [7, 8]. Such NMA receptors are thought to mediate some synaptic excitations in the CNS [8]. Furthermore the relative potency of dissociative anaesthetics and benzomorphans as NMA antagonists parallels their potency in drug discrimination tests [9, 10] and in phencyclidine binding assays [11, 12]. It was therefore of importance to know whether the benz(*f*)isoquinolines were also selective antagonists at this excitatory amino acid receptor. Structure activity studies in both behavioural and binding tests with a series of benz(*f*)-isoquinolines indicated that a bridged compound, LY154045, was considerably more potent than an otherwise similar but unbridged compound, LY154005 [1, 2].

We report here that LY154045, but not LY154005, proved to be a selective NMA antagonist.

MATERIALS AND METHODS

Using the technique of microelectrophoresis, we have performed experiments on pentobarbitone-anaesthetized cats and rats to investigate the effect of benz(*f*)isoquinolines on the excitation of spinal neurones by NMA, quisqualate and kainate (three agonists used to characterize excitatory amino acid receptors [7, 8] and by acetylcholine (ACh)). Full details of the experimental techniques and protocol have been published elsewhere [3]. The central barrels of seven barrel glass microelectrodes were used for extracellularly recording action potentials from single spinal neurones, the firing rates of which were plotted continuously. The outer barrels were used for the electrophoretic ejection of compounds into the region of single neurones and contained the following solutions: *N*-methyl-DL-aspartate Na (NMA, 200 mM, pH 8.1), quisqualate Na (5 mM in 200 mM NaCl, pH 7.8), kainate Na (5 mM in 200 mM NaCl, pH 8.2), acetylcholine Cl (ACh, 200 mM, pH 5.5), ketamine HCl (25 mM in 175 mM NaCl, pH 4.5), dextrorphan tartrate (25 mM in 175 mM NaCl, pH 3.5) and two benz(*f*)isoquinolines, LY154045 HCl (10 mM in 200 mM NaCl, pH 3.5) and LY154005 HCl (10 mM in 30 mM NaCl, pH 3.5). The sixth barrel contained 200 mM NaCl and was used for automatic current balancing.

Ejection of excitants was electronically timed in a regular cycle, electrophoretic currents being adjusted so that each excitant produced a similar submaximal increase in firing rate of the neurone under study. Once reproducible control responses were obtained, the test substance was administered and effects on responses to excitants were expressed as percent change from control firing rate.

RESULTS

Recordings showing the effects of LY154045 were made from a total of 23 cat spinal neurones. Amongst these were 5 Renshaw cells, the other cells being unidentified neurones mainly within the dorsal horn. Ejected with currents of 17 ± 8 nA (mean ± S.D.), LY154045 reduced the responses of cat neurones to NMA by 67 ± 19% (N = 15) whereas responses to quisqualate were reduced by 4 ± 10% (N = 17) and

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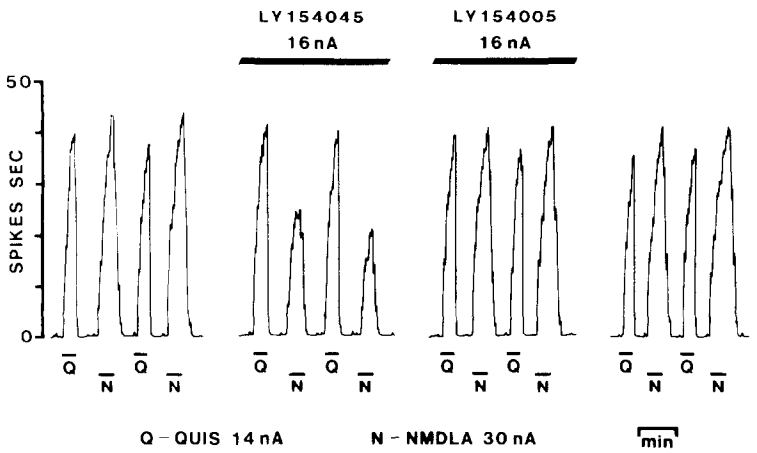

Fig. 1. Comparison of the effects of LY154045 and LY154005 as excitatory amino acid antagonists. On this dorsal horn neurone in a pentobarbitone-anaesthetized cat, all drugs were administered electrophoretically from a multi-barrelled glass microelectrode. The left hand panel shows the control increase in action potential discharge rate in response to quisqualate, 14 nA (Q) and to *N*-methyl-DL-aspartate, 30 nA (N). The centre panels show the responses in the presence of each benz(*f*)isoquinoline as indicated by the bars above the records. Full recovery of responses to the excitatory amino acids was observed after LY154045 and before the ejection of LY154005 (not illustrated) and after the ejection of LY154005, far right panel. Ordinate: action potential discharge rate in spikes/sec; abscissa; time.

those to kainate were enhanced 4 ± 8% (N = 9). On 5 Renshaw cells tested with LY154045 (15 ± 9 nA), ACh actions were unaffected on 3, enhanced 30% on one and reduced 40% on one. This selective antagonism of NMA was dose-dependent and reversible, full recovery usually being observed within 30 min of stopping the ejection of LY154045. Similar effects were also observed on 5 interneurones in pentobarbitone-anaesthetized rats. In this species, LY154045 reversibly reduced or abolished responses to NMA with only marginal effects on responses to quisqualate. Such effects in both cats and rats were obtained in the absence of changes in action potential amplitude or configuration. An example of the action of LY154045 is presented in Fig. 1.

By comparison, LY154005 (19 ± 7 nA), tested on 13 of the same cat neurones reduced responses to NMA by 8 ± 11% and to quisqualate by 3 ± 15%. During these relatively small and variable effects on excitations evoked by amino acids and also by ACh, responses to all excitants were usually affected in parallel (Fig. 1). With only one neurone was there any suggestion of selective NMA antagonism, the response to NMA being reduced by 10% and that to quisqualate being enhanced by 20%. Higher doses of LY154005 were difficult to test because they often led to a non-specific reduction in action potential amplitude but no selective effects on excitations were observed.

Figure 2 summarizes the effects of the two benz-(*f*)-isoquinolines on all cat neurones tested. Comparing the actions of the two benz(*f*)isoquinoline compounds and allowing for the differences in concentration in our electrodes, it would appear that LY154045 is at least 40 times more potent as an NMA antagonist.

In studies comparing the selectivity and potency of LY154045, ketamine and dextrophan, it was found that all three substances produced a similarly selective reduction of NMA responses. With regard to potency, after allowances were made for the different concentrations of antagonists in the microelectrode barrels, we estimate that LY154045 was respectively 5–10 and 3–6 times more potent than ketamine and dextrorphan as an NMA antagonist. An example of records obtained from one neurone showing the

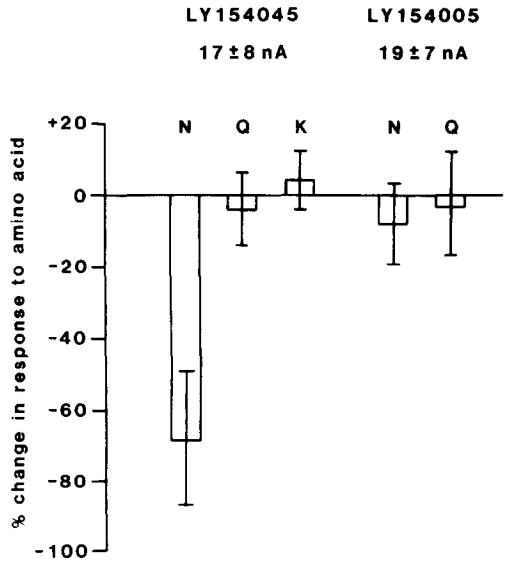

Fig. 2. Histograms showing the mean and S.D. of the effects of LY154045 and LY154005 on responses of cat spinal neurones to excitatory amino acids. LY154045 was tested on the responses of 23 neurones to *N*-methyl-DL-aspartate (N), of 19 neurones to quisqualate (Q) and of 9 neurones to kainate (K). LY154005 was tested on the responses of 13 neurones to *N*-methyl-DL-aspartate (N) and of 10 neurones to quisqualate (Q).

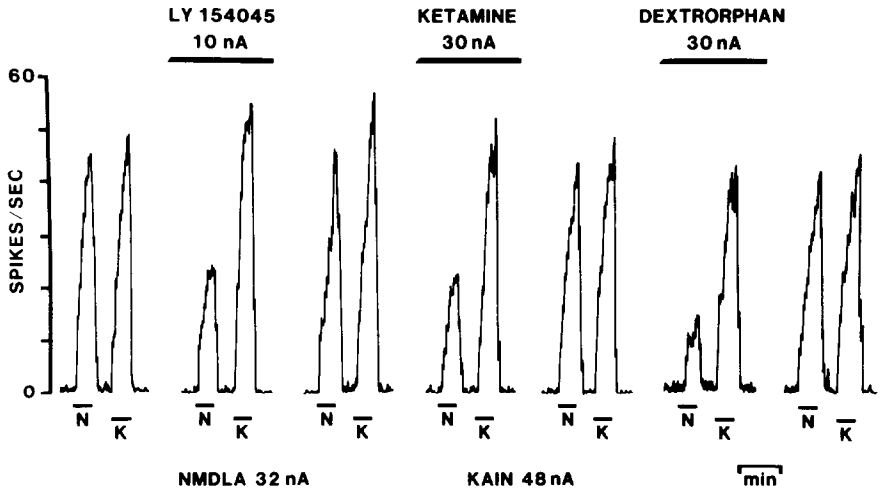

Fig. 3. Comparison of LY154045, ketamine and dextrorphan as excitatory amino acid antagonists. For this cat dorsal horn neurone, the control responses to *N*-methyl-DL-aspartate 32 nA (N) and to kainate 48 nA (K) are shown in the far left panel. LY154045, ketamine and dextrorphan, as indicated by the bar and label above the trace, were each administered for 5 min and recovery from the effect of each antagonist is shown. For potency comparisons it should be noted that LY154045 is diluted 10 mM in 200 mM NaCl whereas ketamine and dextrorphan are 25 mM in 175 mM NaCl. Ordinate: action potential discharge rate in spikes/sec; abscissa: time.

comparison of LY154045, ketamine and dextrorphan as excitatory amino acid antagonists is presented in Fig. 3. It should be noted that for microelectrophoresis the relative concentration of LY154045 was approximately 2.5 times lower than that of ketamine and dextrorphan.

LY154045 was also an effective antagonist of quinolinate, another selective agonist at the NMA receptor [13]. In experiments on 3 spinal neurones LY154045 reduced or abolished the action of quinolinate in parallel with that of NMA whereas the action of quisqualate remained unaffected.

With the possibility in mind that LY154005 might be an antagonist of LY154045, we examined the interaction between these two compounds on amino acid evoked excitations. No evidence was obtained to suggest any mutually antagonistic effects, rather on one or two neurones there appeared to be some very weak synergistic interaction.

DISCUSSION

The selective NMA antagonism demonstrated here with LY154045 is similar to that reported previously for dissociative anaesthetics and sigma opioid benzomorphans [3–6] and to that demonstrated on the same cells with ketamine and dextrorphan, LY154045 being approximately 5–10 and 3–6 times as potent as these latter two compounds respectively. Although not compared directly in the same preparation, we estimate from our present comparisons of LY154045 and ketamine and from our previous studies [3] that LY154045 is somewhat less potent than phencyclidine as an NMA antagonist. The unbridged benz(*f*)isoquinoline, LY154005, was devoid of selective NMA antagonistic properties, with ejecting currents up to those producing a reduction in action potential amplitude.

These results correlate well with those of Zimmerman *et al.* [1] and Mendelsohn *et al.* [2] who showed that LY154045 and not LY154005 generalized toward phencyclidine in drug discrimination studies and displaced phencyclidine binding to CNS tissue. Furthermore our esimates of relative potency of the bridged benz(*f*)isoquinolines and of ketamine and dextrorphan as NMA antagonists agree well with those of Mendelsohn *et al.* [2]. LY154045 can thus be added to the list of behaviourally active drugs which have these two latter properties in common with NMA antagonism. This list includes phencyclidine, ketamine, tiletamine, etoxadrol, dexoxadrol, cyclazocine and *N*-allylnormetazocine [see ref. 6]. LY154005, however, belongs in a list of chemically related substances which have little or no activity in any of the three above experimental tests. This latter list of inactive substances includes levoxadrol, pentazocine, ethylketocyclazocine, morphine and naloxone [see ref. 6]. The strong correlation between binding, electrophysiological and behavioural data suggests that an action at NMA receptors mediating information transfer in the CNS [see ref. 8] underlies some of the subjective effects of the drugs in the former list and, with respect to LY154045, we would predict that this bridged benz(*f*)isoquinoline will have psychotomimetic properties in man.

Our results also imply that NMA receptors and phencyclidine (and LY154045) receptors are functionally related. On both structural and pharmacological grounds, however, it is unlikely that the two receptor sites are identical. LY154045 probably therefore prevents excitation of neurones following electrophoretic ejection of NMA or following synaptic release of a transmitter such as aspartate by interfering with the coupling between activation of the NMA receptor and the conductance change at the associated ionophore.

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