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ORIGINAL INVESTIGATION

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AMPA antagonists differ from NMDA antagonists in their effects on operant DRL and delayed matching to position tasks

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Abstract The effects of NBQX (1.56–7.5 mg/kg, IP), a competitive antagonist at the AMPA type of glutamate receptor, were studied in two operant behavioural paradigms, differential reinforcement of low response rates (DRL), and delayed matching to position (DMTP), which have been shown to be sensitive to the antagonists of the NMDA type of glutamate receptor. Additionally, the non-competitive AMPA antagonist, GYKI 52466 (7.5–15 mg/kg, IP), was studied in the DRL procedure. As a positive control, the non-competitive NMDA antagonist, MK 801 (0.0125–0.1 mg/kg, IP) was studied in both procedures. During performance of the DRL schedule, MK 801 increased response rates in a dose dependent manner, and decreased the number of reinforcers obtained. The increase in response rates could be attributed to both a shift in the median inter-response time (IRT) to shorter intervals, and to a marked, dose dependent increase in the occurrence of bursts of responses (responses occurring within 3 s of a previous response). In contrast, NBQX and GYKI 52466 both decreased response rates in a dose dependent fashion, and did not shift the distribution of the IRTs, or increase the occurrence of burst responding. In the DMTP procedure, accuracy of matching decreased with increasing delay (up to 30 s, between presentation of sample and opportunity to respond). NBQX disrupted responding at a dose of 7.5 mg/kg, but lower doses were ineffective in influencing accuracy of performance of the discrimination. In contrast, MK 801 (0.1 and 0.2 mg/kg) reduced accuracy of matching at all delays, while tending to increase the speed of responding. These data demonstrate differences in the effects of AMPA and NMDA antagonists on performance of well trained operant behaviour.

Key words NBQX · GYKI 52466 · MK 801 · DRL responding · Delayed matching · AMPA antagonists · Glutamate receptors

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Introduction

Glutamate is the major excitatory transmitter in the central nervous system of vertebrates (Curtis et al. 1959; Cotman et al. 1987), and acts through a number of receptor types which gate ion channels, or are coupled to G-proteins. Of the receptors serving to gate ion channels, three subtypes have been characterised pharmacologically, and have been named after their preferred agonists, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydro-5-methyl-4-isoxazolpropionic acid (AMPA; previously quisqualate), and kainate receptors, respectively (Watkins et al. 1990). The behavioural pharmacology of NMDA antagonists is now well described, largely because of the availability of drugs acting at several sites on the NMDA receptor complex. Thus, compounds have been described which act as competitive antagonists at the glutamate binding site (e.g. AP5 (Davies et al. 1981), or CGS 19 755 (Bennett et al. 1989), as antagonists of the glycine modulatory site on the receptor complex (e.g. HA 966; Carter 1992), as blockers of the Ca^{2+} channel gated by NMDA agonists (e.g. MK 801: Wong et al. 1986), and as antagonists at a polyamine site on the complex (e.g. eliprodil; Carter et al. 1988). All such compounds act to reduce the ability of glutamate to induce Ca^{2+} flux through membranal channels, and to induce neuronal excitation. Since glutamate-induced Ca^{2+} influx through NMDA gated channels has been implicated in several neurodegenerative disorders, NMDA receptor blockers hold great therapeutic promise. Furthermore, NMDA

receptors have been suggested to play important roles in synaptic plasticity in long term potentiation, and in learning. Although some differences in the behavioural pharmacology of NMDA competitive and non-competitive antagonists have been described (e.g. Cole et al. 1993), in general NMDA receptor antagonists have wide ranging behavioural effects, including disrupting learning in tests of spatial learning (Morris 1989) as well in other learning tasks (Danysz et al. 1995), and anti-punishment, and other anxiolytic-like properties (Stephens et al. 1986; Wiley and Balster 1992). In these latter respects, NMDA antagonists may resemble benzodiazepines (Balster and Wessinger, 1983; Stephens et al. 1991a), and indeed, in drug-discrimination procedures, rats trained to discriminate benzodiazepines from vehicle generalise to NMDA antagonists (Bennett et al. 1987). Consistent with such observations, effects of the NMDA channel blocker MK 801 have been demonstrated in a DRL procedure (Welzl et al. 1991; Sanger 1992), and on performance of a spatial delayed matching task (DMTP) by rats (Cole et al. 1993), tasks which have been shown to be sensitive to the effects of benzodiazepines (Sanger and Blackman 1975; Cole and Hillmann 1994; Stephens and Voet 1994).

Considerably less information is available on the behavioural effects of drugs acting as antagonists of AMPA receptors, although these receptors, which usually gate Na^+ channels, are of more importance than NMDA receptors in the maintenance of excitatory fast transmission in the mammalian CNS. This situation has arisen because, until recently, specific antagonists of AMPA receptors have not been readily available, and have shown poor brain penetration. More recently, both competitive antagonists at the AMPA receptor (e.g. NBQX; Sheardown et al. 1990), and substances acting at an as yet poorly characterised modulatory site (e.g. GYKI 52466; Zorumski et al. 1993) have become available. These compounds, too, have been shown to possess neuroprotective properties in models of cerebral ischaemia (Sheardown et al. 1990; Smith et al. 1991). However, little is known about the effects of AMPA antagonism on behaviour. We have recently reported antispastic properties of NBQX (Turski et al. 1992) and antipunishment properties when the compound was administered IP 5 min (Turski et al. 1992) but not 30 min (Stephens et al 1991a) before testing. These initial findings suggest a similarity between AMPA antagonists and NMDA antagonists in their behavioural pharmacology, and an overlap with the pharmacology of benzodiazepines. On the other hand, Swedberg et al. (1995) report that NBQX does not share discriminative stimulus properties with the NMDA antagonist, phencyclidine. The present paper explores further the effects of AMPA antagonists in two operant procedures sensitive to benzodiazepines, and compares them with the action of the NMDA receptor non-competitive antagonist, MK 801.

Materials and methods

Animals

The subjects were male Wistar rats obtained from the Department of Animal Breeding, Schering AG, and weighing 200 g at the outset of training. The rats were singly housed, and maintained in animal holding facilities at a temperature of 21°C and relative humidity of 55%, with a dark/light cycle of 12 h (lights on at 0700 h). Motivation for responding was maintained by restricting access to food to the reinforcements obtained during sessions, and a daily ration of 15–20 g Sniff rodent diet (Sniff Versuchstierdiät, Soest, Germany) given following the operant session, and at weekends. All experiments were carried out under German legislation on animal experimentation.

Differential reinforcement of low response rates

Eight rats were trained to press a lever in standard operant chambers (Coulbourn Instrument, Lehigh Valley, Pa., USA) to obtain 45 mg pellets of food (Bioserve, Frenchtown, N.J., USA). Reinforcement contingencies were controlled, and data collected using Compaq personal computers running OPN software (Spencer and Emmett-Oglesby 1985) to control MedAssociates (MedAssociates, East Fairfield, Vt., USA) interfaces. After the rats were trained in initial sessions to operate a lever to obtain food pellets (one pellet for each lever press), responding was maintained by a DRL schedule of reinforcement according to which only responses occurring a certain time after the previous response were reinforced with food. Initially the interval between responses (inter-response time; IRT) to obtain food was set at 5 s (two sessions), followed by five sessions with a 10 s IRT requirement, and a prolonged training (35 sessions) with a required IRT of 15 s. Training sessions occurred initially three times daily, and were reduced to daily sessions after 15 sessions of DRL15 s. The rats were tested 5 days each week at approximately 0800 hours, and sessions were of 45 min duration. When performance on this schedule had become stable, drug testing started. Drugs were administered twice weekly, each individual drug being tested over a dose range administered according to a Latin square design employing three doses and vehicle. Groups of eight rats were used for each dose. On completion of the dose range for one drug, the animals were used successively for the other drugs in the series, using the same design. All animals received all drugs in the same order of testing (NBQX, GYKI 52466, MK 801). Drug testing took place on Tuesday and Friday of each week, the intervening week days serving as retraining days.

Data analysis and statistics

Total number of responses during a session, and total number of reinforcements were recorded, and the data from the doses tested under the Latin square design subjected to one way, repeated measures ANOVA (factor: dose) for each drug. Post hoc Neumann-Keuls tests were employed to identify differences between individual doses and vehicle. Additionally, responses were classified according to the IRT at which they occurred, and totalled in 3-s bins of 0–3 s, 3–6 s, 6–9 s, etc, up to 30–33 s. A last bin was allotted to collect all responses occurring at IRTs greater than 33 s. Numbers of responses occurring in the first bin were analysed separately using Friedman analyses of variance.

The remaining data were used to calculate for each rat and drug dose, the number of responses (n) occurring within each bin. Since we found a strict relationship between the variance within a bin, and the bin number (late bins had less variance than early bins), the assumptions for analysing the data according to a repeated measure design ANOVA were not met. On the other hand, there was

a strong nonlinear relationship between response probability and bin number, which could be described by the Weibull distribution (Ratkowsky 1990). Cumulated probability curves were thus fitted to the data for each individual rat and treatment (Stephens and Voet 1994). The probability curves are a sigmoid function in which the inflection point of the curve represents the median IRT. The median IRT was thus calculated for each rat and dose, and the slope of the linear regression describing the relationship between the shift in the IRT and drug dose compared to the null hypothesis (i.e. no effect of treatment). Occasionally, the drugs severely depressed responding, so that meaningful data could not be collected. These data points were treated as missing values in the ANOVA, and the degrees of freedom adjusted appropriately.

Delayed matching to position

Twenty-one rats served as subjects, of which 12 were assigned to the NBQX experiment, and nine to the MK 801 experiment. Eight operant chambers similar to those described above, and using identical hard and software to control behavioural contingencies and record data were used. Each chamber was equipped with two retractable levers and a food tray into which food pellets could be delivered. A photocell assembly (Medlab Associates, East Fairfield, Vt. USA) was installed horizontally across the entrance to the food tray, to enable nose pokes to be detected; cage dividers were not used. The rats were trained according to Cole et al. (1993) to operate either lever to obtain food, before being trained to perform the matching rule. During this phase, each trial began with the insertion of the lever (sample) into the chamber. When the rat pressed this lever it was retracted into the front wall, and the tray light illuminated. The first nose poke into the food tray caused the tray light to be extinguished, and both levers to be inserted into the chamber, without delay. If the rat now pressed the correct lever (i.e. that which had been presented as sample) it was reinforced with a food pellet. Both levers were then retracted and a 10-s intertrial interval (ITI) ensued before the start of the next trial. If the rat failed to respond within 5 s, or pressed the wrong lever, no food was delivered, but the levers retracted, followed by the 10-s ITI. Each session consisted of a maximum of 128 trials, and had a maximum duration of 60 min. After the rats had achieved stable performance on this schedule, they were introduced to a simple version of the delayed matching procedure, with delays of either 0 or 5 s. Thereafter, the rats were trained using the final parameters. Each session consisted of a maximum of 128 trials, with four delays: 0, 5, 15 and 30 s (32 trials per delay). The order of the delays and sample presentations was random but counterbalanced. Training continued on this schedule until performance was stable.

Data analysis

The number of correct and incorrect responses were used to calculate the percent accuracy measure for each delay. In addition, two behavioural measures of motoric ability or sedation were recorded: the total number of nose pokes per session, and the latency to make a matching response at each delay. Only the data from rats responding on at least 50% of trials at every delay were included in the analyses. Data from rats failing to meet this criterion were treated as missing values in subsequent analyses, with the loss of the appropriate degrees of freedom. The accuracy and the latency data were analysed using a within subjects, repeated measures ANOVA, with repeated measures on the factors Dose and Delay. The number of nose pokes was also analysed using a repeated measures ANOVA, with one factor, Dose. Following identification of significant main effects or interactions in the ANOVA, specific comparisons were made using post hoc Neuman-Keuls tests.

Drugs

NBQX was obtained from Novo Nordisk, Maloev, Denmark, GYKI 52466 from the Institute for Drug Research, Budapest, Hungary, and MK 801 from MSD, Terlings Park, England. NBQX and GYKI 52466 were dissolved in 0.1 N sodium hydroxide, the pH adjusted to neutrality with 0.1 N hydrochloric acid, and made up to volume with 5% glucose solution, and administered intraperitoneally. In the DRL task NBQX was given 15 min, and GYKI 52466 10 min before each session; in the delayed matching task, NBQX was given 30 min before testing. MK 801 was dissolved in 0.9% saline and given 30 min before testing.

Results

Differential reinforcement of low rate

The DRL 15-s schedule maintained response levels of between 180 and 200 per 45-min session, with reinforcement frequencies between 85 and 102 per session. Figure 1 illustrates that over the tested dose range, NBQX decreased the response rate [$F(3, 21) = 15.2$, $P < 0.001$] and the number of reinforcements [$F(3, 21) = 42.5$; $P < 0.001$], post hoc Newman-Keuls tests indicating significant effects of doses above 3.5 mg/kg ($P < 0.01$). A similar pattern was observed with GYKI 52466 (Figs 2 and 3) which gave rise to dose related decreases in response rate [$F(3, 18) = 9.4$; $P < 0.001$] and reinforcements [$F(3, 18) = 7.4$; $P < 0.01$]. Post hoc analyses revealed significant effects on both parameters of both the 11 and the 15 mg/kg dose ($P < 0.001$). Since GYKI 52466 possesses a short half-life, the data was also analysed by dividing the session into three 15 min blocks, in anticipation that effects might have been observable at short post-injection intervals which were diluted out by the data at longer intervals. No effects were seen at any post injection interval for the 7.5 mg/kg dose, but Fig. 2 shows that the drug effects observed for the session for the 11 mg/kg dose were attributable largely to effects within the first two 15-min blocks. Figure 4 shows that MK 801 significantly increased the total number of responses [$F(4, 28) = 6.5$; $P < 0.001$] and decreased reinforcements [$F(4, 28) = 31.8$; $P < 0.001$], post hoc tests showing significant effects of only the highest dose (0.1 mg/kg; $P = 0.001$) on response rate, but of all doses tested on the number of reinforcements.

The figures also show the frequency distribution of IRTs under vehicle and drug treatment. Under vehicle treatment, the mode of the IRT frequency curve occurred in the sixth bin (15–18 s), indicating accurate responding. MK 801 (Fig. 4) shifted the mode of the IRT distribution to shorter IRTs [shown by shifts in the inflection point of the Weibull distribution, [$F(1, 38) = 45.3$; $P < 0.0001$; Fig 5], post hoc analyses indicating significant effects of dose. Neither NBQX (Figs 1 and 5) [$F(1, 22) = 3.13$; ns] nor GYKI 52466 (Figs 2 and 5) [$F(1, 25) = 3.52$; ns] reliably shifted the

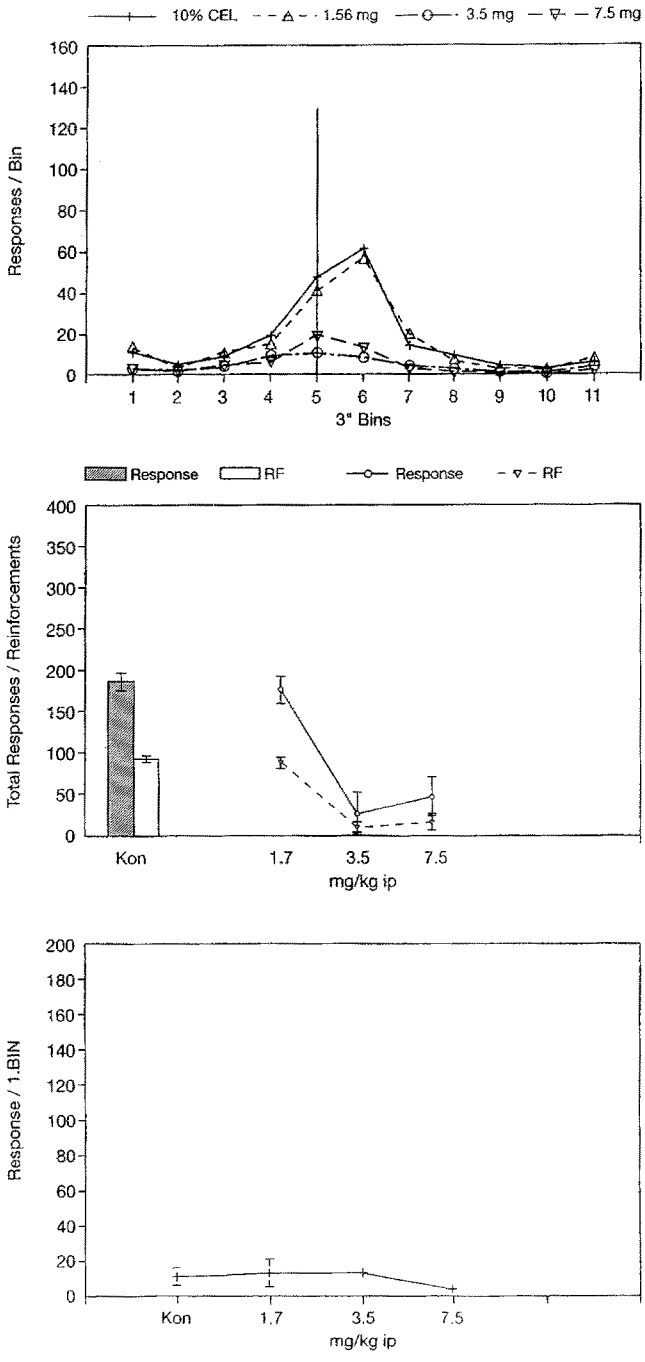

Fig. 1 The effects of NBQX on DRL performance by rats. The *upper panel* shows the total numbers of responses emitted during the session in each of the 3-s bins following a previous response during performance of the DRL 15-s schedule. Bin 11 contains all responses emitted with IRTs greater than 33 s. The *middle panel* shows the effects on the total numbers of responses and reinforcers (*RF*) obtained during the session; *bars* indicate standard deviations. The *lower panel* indicates the effects of NBQX on the number of responses emitted in the first 3-s bin following a previous response. Doses of NBQX are indicated above the upper panel. *CEL* or *Kon* indicate performance under vehicle control (Cremophor EL vehicle)

distribution of the IRTs, but both compounds flattened the distribution at higher doses.

Inspection of Fig. 4 reveals that MK 801 also increased the number of responses in the first bin (Friedman analysis of variance, $X^2 = 19.73$, $df = 4$, $P < 0.01$), an effect which post hoc tests indicated was significant at the doses of 0.05 and 0.1 mg/kg. Neither NBQX nor GYKI 52466 had statistically reliable effects on burst responding, either when the data was considered over the entire session, or for only the first 15 min.

Delayed matching to position

The highest dose of NBQX (7.5 mg/kg, IP) severely disrupted performance in nine out of 12 rats tested so that they did not respond on at least 50% of trials (in fact they failed to respond on any trial). For this reason, no analyses were carried out on data collected with this drug dose. Figure 6A shows the effects of the remaining two doses of NBQX (1.7 and 3.5 mg/kg) on matching accuracy for all 12 animals tested. Accuracy declined with increasing delay [$F(3, 33) = 18.9$; $P < 0.0001$], but there was no significant main effect of Dose [$F(2, 22) = 0.1$; ns]. Latencies to respond increased with increasing delay between sample presentation and matching opportunity [$F(3, 33) = 9.7$; $P < 0.001$), but the doses of NBQX used also failed to affect latency to respond (Fig. 5B) [$F(2, 22) = 1.49$; ns]; the number of nose pokes was weakly reduced by NBQX (Fig. 5C) [$F(2, 22) = 3.53$; $P < 0.05$]. Post hoc Neumann Keuls tests revealed that this effect was attributable to differences between the 3.5 mg/kg dose, and vehicle.

Following treatment with the highest dose (0.1 mg/kg) of MK 801, one of the nine rats failed to respond on 50% of trials. These data points were treated as missing values in the analyses. Figure 7A shows that matching accuracy declined with increasing delay [$F(3, 24) = 53.2$; $P < 0.001$] and was also impaired by MK 801 [Main effect of Dose, $F(4, 32) = 51.6$; $P < 0.001$]; there was also a significant Interaction term [$F(12, 96) = 5.2$; $P < 0.001$] and inspection of Fig. 7A suggests that the impairment was greater at longer delays. Post hoc Neuman-Keuls tests indicated that only the highest dose gave rise to significant ($P < 0.05$) effects on accuracy at any delay, so that the significant interaction term may be attributable to the highest dose having a greater effect at longer delays.

Panel B of Fig. 7 shows that response latencies increased with increasing delay between sample presentation and matching opportunity [$F(3, 21) = 22.0$; $P < 0.001$], but there was no main effect of Dose [$F(4, 28) = 1.7$; ns]. A significant interaction term [$F(12, 84) = 5.2$; $P < 0.001$] suggests that the effects of delay on latency varied across the doses. Figure 7C indicates that MK 801 also increased the number of nose pokes [$F(4, 32) = 3.2$; $P < 0.05$].

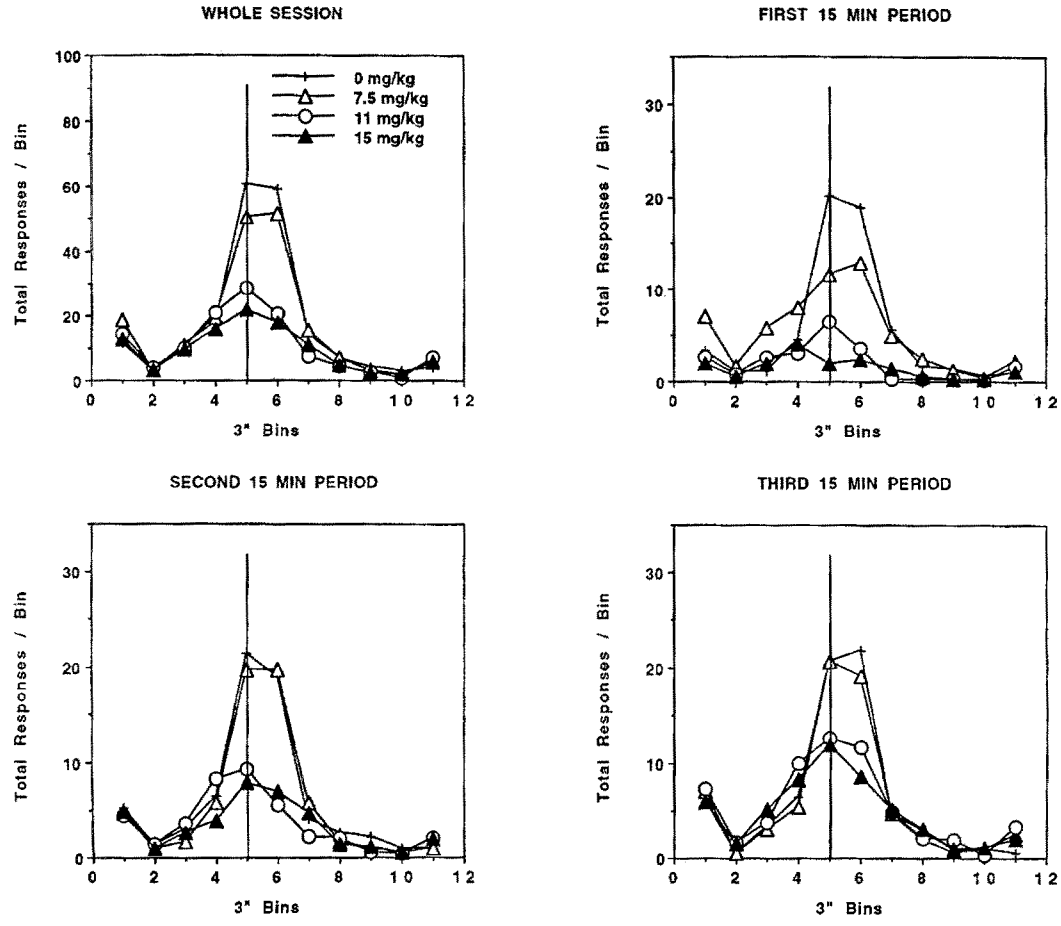

Fig. 2 The effects of GYKI 52466 on DRL performance by rats. The panels show the total numbers of responses emitted during the complete session (*upper left*), or for the first 15 min (*upper right*), second 15 min (*lower left*) or third 15 min of the session (*lower right panel*) in each of the 3-s bins following a previous response during performance of the DRL 15-s schedule. Bin 11 contains all responses emitted with IRTs greater than 33 s

Discussion

The experiments reported here reveal that the behavioural pharmacology of two AMPA antagonists, one (NBQX) acting competitively at the AMPA binding site, and the other (GYKI 52466) at a modulatory site, differs considerably from that of an NMDA non-competitive antagonist, and from the profile of competitive NMDA antagonists as described in the literature. Firstly, the NMDA antagonist, MK 801, in confirmation of previous findings (Welzl et al. 1991; Sanger 1992), increased response rates of rats performing a DRL schedule, both by increasing the tendency to produce bursts of responses, and by shifting the IRT distribution to the shorter IRTs, resulting in a decrease in the number of reinforcements. These effects were marked, and the increase in burst responding was larger

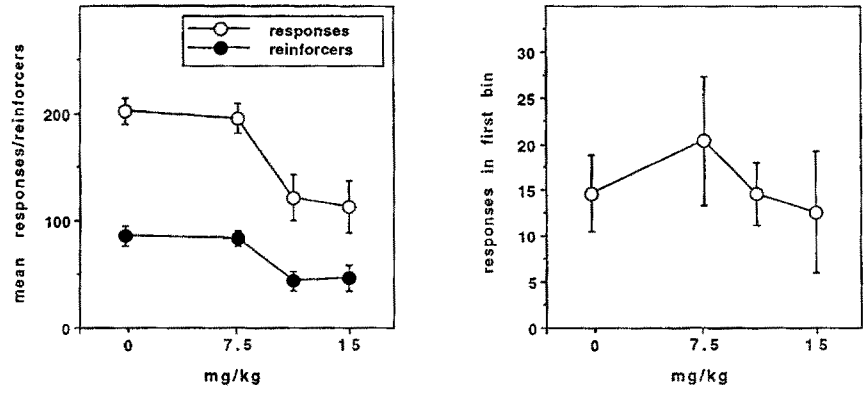

Fig. 3 The effects of GYKI 52466 on DRL performance by rats. The *left panel* shows the total numbers of responses emitted and reinforcers obtained during the 45-min session at each of the doses. The *right panel* indicates the numbers of responses in the first 3-s bin following a previous response, a measure of "burst" responding

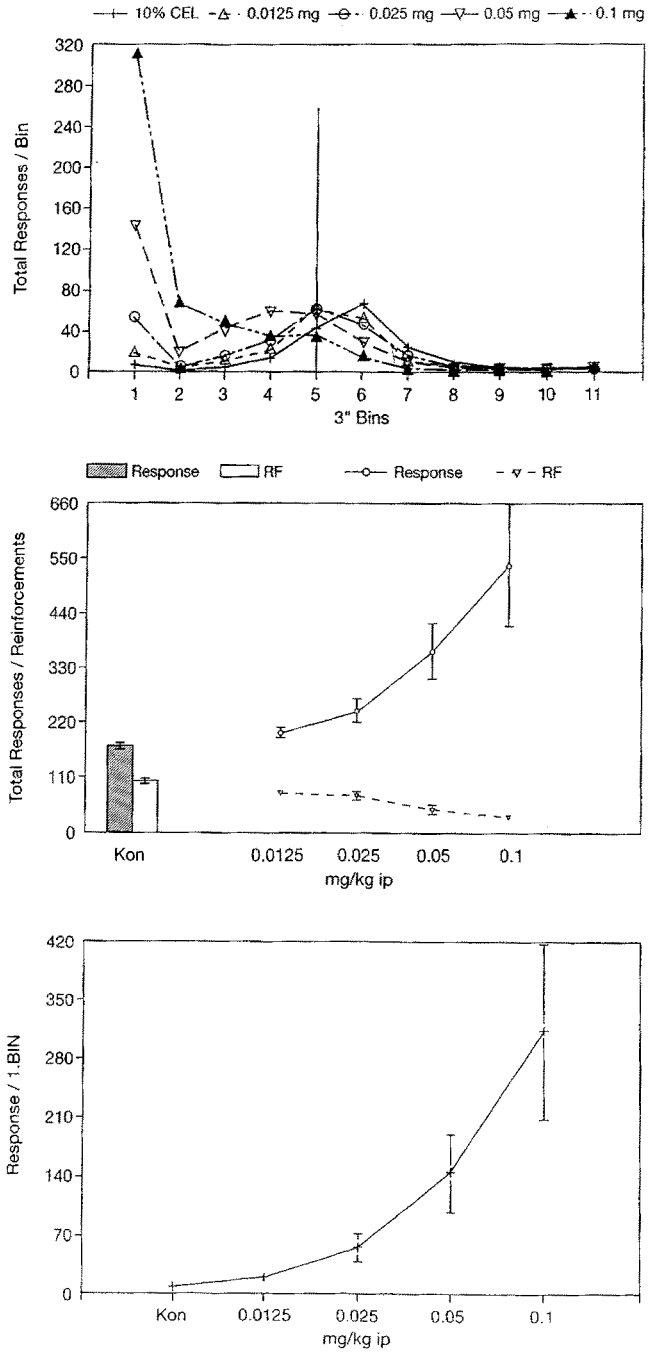

Fig. 4 The effects of MK 801 on DRL performance by rats. The *upper panel* shows the total numbers of responses emitted during the session in each of the 3-s bins following a previous response during performance of the DRL 15-s schedule. Bin 11 contains all responses emitted with IRTs greater than 33 s. The *middle panel* shows the effects on the total numbers of responses and reinforcers (*RF*) obtained during the session; *bars* indicate standard deviations. The *lower panel* indicates the effects of MK 801 on the number of responses emitted in the first 3-s bin following a previous response. Doses of MK 801 are indicated above the upper panel. *CEL* or *Kon* indicate performance under vehicle control (Cremophor EL vehicle)

than those we have seen previously with drugs acting at benzodiazepine receptors (Stephens and Voet 1993). Neither NBQX nor GYKI 52466 showed similar effects. This could not be attributed to a failure of these compounds to enter the brain since both drugs reduced the rates of lever pressing at active doses without altering the IRT distribution. Furthermore, the absence of a specific effect on IRT distribution is unlikely to be due to the rapid metabolism of the drugs since dividing the 45-min session into three 15-min parts for separate analyses indicated that, although the effects of GYKI 52466 were less pronounced in the last 15-min period, there was no effect of the compound on the IRT distribution at any period. Thus, at doses which did not disrupt lever pressing, the AMPA antagonists, both competitive and non-competitive, had no effects on performance of the DRL schedule. These results suggest that blockade of AMPA-receptor mediated transmission has quite different effects on behaviour controlled by a DRL 15-s schedule from blockade of NMDA receptor-mediated transmission. Although we tested only the non-competitive NMDA receptor antagonist MK 801 in the present experiment, a number of reports indicate that the effects of non-competitive and competitive antagonists of NMDA receptors on DRL performance resemble each other in several paradigms (Balster and Baird 1979; Poling et al. 1981; Welzl et al. 1991; Hudzik and Slifer 1992; Sanger 1992). Thus the channel blockers MK 801, phencyclidine and memantine, as well as the competitive antagonist CGS 19755, have been reported to increase burst responding, and reduced median IRT values, as did MK 801 in the present experiment.

A consistent effect of both NBQX and GYKI 52466 in the present experiments was the depression of rates of lever pressing. This, too, stands in contrast to the effects of NMDA antagonists on operant responding, which while not always consistent, appear at appropriate doses to increase response rates (Boast et al. 1988; Sanger and Jackson 1988; Tonkiss et al. 1988; Bennett et al. 1989), though higher doses disrupt such behaviours. It cannot be entirely ruled out that the reason why effects of NBQX and GYKI 52466 on timing behaviour were not seen, was that the behaviourally disruptive effects of the AMPA antagonists occurred at doses lower than those at which more specific behavioural effects occur. We have previously reported that muscle relaxant effects of NBQX are to be observed at doses as low as 0.05 mmol/kg (equivalent to 16.8 mg/kg) after IP administration, probably as a consequence of its effects on monosynaptic spinal reflexes (Turski et al. 1992) and it is possible that these effects on motor performance masked the expression of more subtle behavioural effects.

The effects of drugs on performance of DRL responding have been discussed within several theoretical contexts. Some researchers (e.g. Gray 1981) have emphasised that drugs such as benzodiazepines which act on a behavioural inhibition system to disinhibit behaviours which are normally inhibited in the presence of signals of nonreward, or punishment, can be

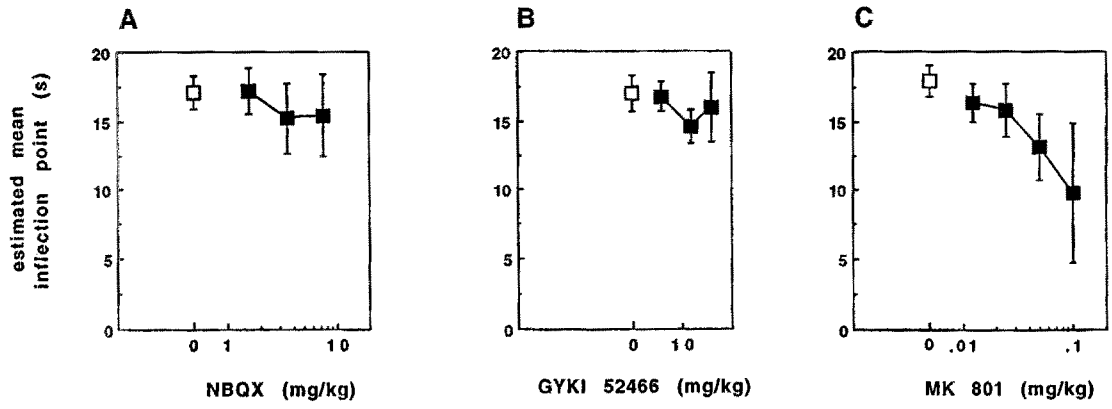

Fig. 5A–C Effects of NBQX (A), GYKI 52466 (B) and MK 801 (C) on the inflection point of the fitted Weibull distribution (as an estimate of the median inter-response time) in performance of the DRL 15-s schedule. *Bars* indicate standard deviations

expected both to increase burst responding, and to shift the IRT distribution to shorter intervals. The absence of effects of NBQX and GYKI 52466 in these parameters would thus argue against them influencing the behavioural inhibition system at doses which do not interfere with behavioural output, and predict that such compounds would not have anxiolytic activity. Such a conclusion is consonant with those of Stephens et al. (1991a; but see Turski et al. 1992) and Swedburg et al. (1995).

Others have discussed DRL performance in terms of the animal's ability to estimate elapsed time (e.g. Sanger and Blackman 1975; Wogar et al. 1992). A variation on this idea points out that efficient timing requires an element of temporary memory since the subject must somehow store the timing of the previous response (Tonkiss et al. 1988). In a test of the effects of an AMPA antagonist on performance of a task specifically requiring storage of information in a temporary memory store, we also investigated the ability of NBQX to disrupt performance of a delayed matching to position paradigm. We have previously demonstrated an effect of NMDA antagonists on performance of a DMTP task, the competitive antagonist CPP decreasing accuracy of discrimination in a delay dependent fashion, while MK 801's effects were less specific, and disrupted discrimination even when no delay was interposed between presentation of the sample, and the opportunity to perform the discrimination (Cole et al. 1993). In the present experiments we were able to replicate these findings with MK 801, and to demonstrate that a lower range of doses than that used in our previous experiment was ineffective in the same task. In contrast to MK 801 and to our previous findings with the competitive NMDA antagonist, CPP, the AMPA antagonist showed no effects at doses (1.75 and 3.5 mg/kg) which did not disrupt responding; a higher dose, 7.5 mg/kg, depressed behaviour so severely that no meaningful interpretation of data from this dose was possible. It cannot be entirely ruled out that the dose-response relationship for NBQX in the DMTP task is very steep, and that a dose intermediate between 3.5 and 7.5 mg/kg may have given rise to effects on accuracy of matching, or milder sedation. In such a case, it might be expected that individual animals might have shown such effects at the neighbouring doses to the hypothetically effective one. Inspection of data from individual animals for the 3.5 and 7.5 mg/kg doses showed no such tendencies. The results from this experiment, then, again demonstrate a dissociation of the effects of NMDA and AMPA antagonists.

In as much as the delay-dependent decrement in accuracy of discrimination performance of the DMTP task reflects a function of working memory, these findings suggest that NMDA, but not AMPA receptors are critical in this function. However, as we discuss elsewhere (Cole et al. 1993), the interpretation of drug effects in the DMTP procedure is not clear if drugs have effects even at zero delay. In these circumstances, drug effects are equally ascribable to drug-induced deficits in attention or perception, or even motor performance. The fact that at doses which did not disrupt lever pressing, NBQX had no effects at zero delay, implies that these doses also did not interfere with the attentional or perceptual requirements of the task.

It is of interest that the kinds of drug which disrupt DRL and DMTP (benzodiazepines, NMDA antagonists, scopolamine) are also those which impair passive avoidance performance if given during, or immediately following the acquisition phase of such experiments. Since AMPA antagonists are ineffective in disrupting DRL, DMTP and avoidance conditioning at doses which are consistent with responding, it appears that NMDA receptors, but not AMPA receptors play an important role in at least two types (or stages) of memory – short term working memory and consolidation of information into longer term stores. A role of NMDA receptors in a form of working memory can also be inferred from findings that NMDA antagonists such as CPP impair performance in the radial maze in that they increase the probability of rats revisiting baited (but not never-baited) arms within a trial (Danysz et al. 1988; Ward et al. 1990; Lyford and

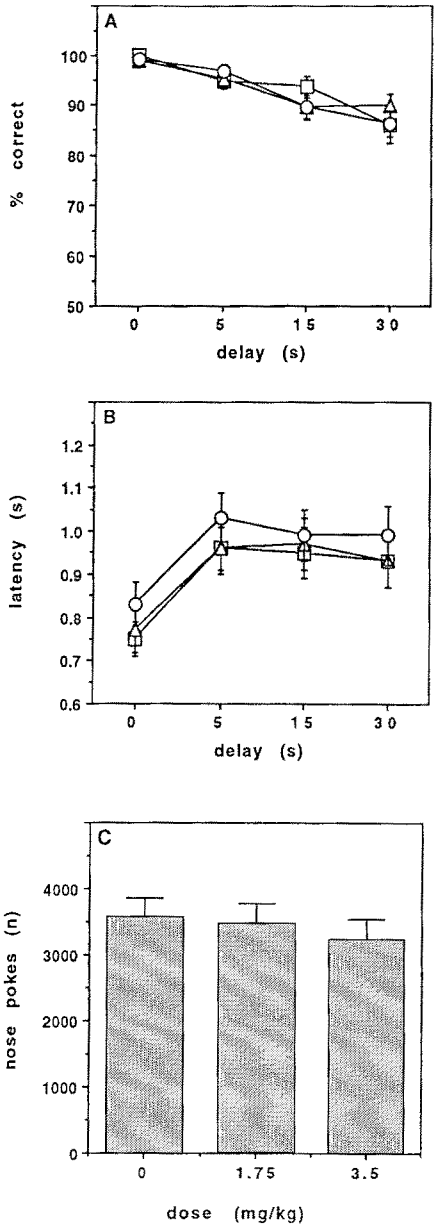

Fig. 6A–C Effects of NBQX on the accuracy of discrimination in the delayed matching to position procedure (*upper panel*) and on two measures of sedation, the mean latency to respond (*middle panel*) and the numbers of nose pokes made in the delayed matching session. *Bars* indicate standard errors of the mean. –□–, 0 mg/kg; –△–, 1.75 mg/kg; –○–, 3.5 mg/kg

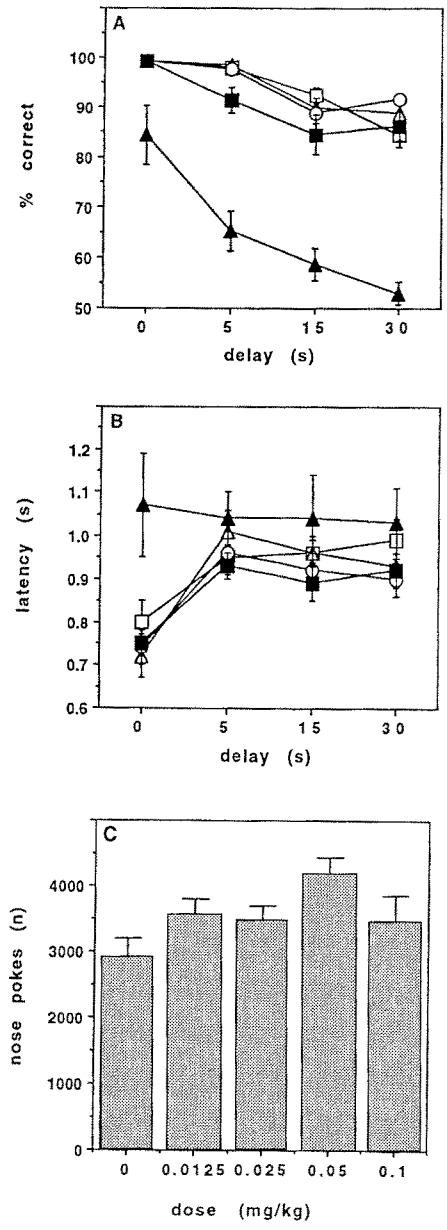

Fig. 7A–C Effects of MK 801 on the accuracy of discrimination in the delayed matching to position procedure (*upper panel*) and on two measures of sedation, the mean latency to respond (*middle panel*) and the numbers of nose pokes made in the delayed matching session. *Bars* indicate standard errors of the mean. –□–, vehicle; –△–, 0.0125 mg/kg; –○–, 0.025 mg/kg; –■–, 0.05 mg/kg; (–▲–), 0.1 mg/kg

Jarrard 1991). It may be important that AMPA antagonists have not been reported to impair performance of maze tasks requiring working memory (Danysz et al. 1995).

The failure in the present experiments of AMPA antagonists to induce burst responding, or to shorten IRTs in the DRL paradigm, or to disrupt performance of the delayed matching procedure, implies that intact AMPAergic transmission is not essential for such processes. This is surprising, since AMPA receptor-mediated transmission accounts for most fast synaptic transmission in the CNS, and it is difficult to imagine that such transmission is not involved in these higher functions. Although our results might be taken to suggest that AMPA receptor-mediated mechanisms do not play an essential role in such processes, it cannot be ruled out that the doses which we could test were unable to give rise to sufficiently high concentrations of drug in those parts of the brain involved in these behaviours. Infusion of drug locally into specific brain areas where it is unlikely to give rise to motor impairment may avoid such problems, though presently available AMPA

antagonists are not ideal for intracranial application, since they are not readily soluble under physiological conditions.

There have been a number of suggestions recently that AMPA receptor mediated transmission may be important in the expression, but not the acquisition of several conditioned behaviours. In behavioural experiments, NBQX or CNQX, a less specific AMPA antagonist, given intracranially post-training have been reported to prevent the consolidation (Danysz and Wroblewsky 1989; Flood et al. 1990; Jerulinsky et al. 1992; Izquierdo 1994), or expression (Liang 1994; Quillfeldt et al. 1994) but not the acquisition (Parada et al. 1992; Misztal and Danysz 1995) of avoidance conditioning (see Danysz et al. 1995 for a review). Similar observations have been made for fear-potentiated startle (Kim et al. 1993), and in place preference conditioning for morphine and amphetamine (Layer et al. 1993), and cocaine (Cervo and Saminin 1995), though some of these experiments must be regarded as incomplete since the appropriate control groups have not always been studied. Nevertheless, at face value these data suggest that while NMDA, but perhaps not AMPA receptors are important in mediating the plasticity underlying conditioning, AMPA receptors are necessary for the consolidation or expression of behavioural modifications arising from such plastic changes. It may be of relevance that AMPA antagonists also prevent the expression, but not the induction of LTP, at least in vitro (see Izquierdo 1994; Danysz et al. 1995), whereas NMDA antagonists are effective in blocking acquisition, but not expression (see, e.g. Danysz et al. 1995). A similar suggestion has been made for the processes underlying kindling of epileptic states in which NMDA, but not non-NMDA receptors apparently play a key role in the development of kindling, but in which non-NMDA receptors are involved in the expression of kindled seizures (Durmuller et al. 1994). It might therefore be speculated that whereas NMDA receptors are intimately involved in plastic processes underlying storage of memories, AMPA receptors are important in the use of such memories for the performance of tasks depending on them. If this is the case, AMPA antagonists might offer a new pharmacological tool for studying retrieval mechanisms.

The temporary storage and use of information necessary for performance of either DRL, or DMTP is at first sight different from the information storage involved in fear or drug-place conditioning and might be expected to be based on different neurobiological systems. DRL and DMTP have certain requirements in common, which differ from both passive avoidance and place conditioning. Firstly, the latter have discrete phases for the acquisition of information (conditioning phase) and for the application of the acquired knowledge (test phase); this might be formally similar to the training phase of DRL and DMTP in which the animals learn the requirements of the tasks, which they then apply during the performance phase. Additionally, however, DRL and DMTP procedures run as a continuous series of discrete trials within a session and performance of DRL and DMTP thus requires a temporary storage of information as envisaged in theories of working memory. Additionally, performance within any given trial in the DRL and DMTP procedures can be expected to be subject to interference from previous trials within the session, whereas interference effects across trials are unlikely to contribute to performance decrements in the conditioning tasks.

We have argued previously (Stephens et al. 1991b) that in the case of benzodiazepines, the amnestic effects may arise from a slowing of processing within a limited capacity working memory, resulting in loss of information before consolidation can be completed. The effects of NMDA antagonists on learning processes are consistent with NMDA receptors being involved in some limited resource, as described by Kahneman's (1973) characterisation of attention as a limited reserve of processing capacity necessary for carrying out mental work, or Baddeley's (1981) concept of working memory as a limited capacity system for temporary storage and manipulation of recent input. The implication is that drugs (like glutamate antagonists or benzodiazepines) which slow processing in such a system would induce amnesia if the system was subjected to a high load (massed trials) but not to a light load (spaced trials), a result reported by Upchurch and Wehner (1990) for the amnestic effects of the NMDA competitive antagonist CPP in the Morris water maze.

A reduction in the capacity of a working memory system (or slowed processing within a working memory system of limited capacity) would also explain the effects of NMDA antagonists (and BZs) in delayed discriminations (Tan et al. 1989; Pontecorvo et al. 1991; Cole et al. 1993), or delayed alternation (Tonkiss and Rawlins 1991) in which interference from recent prior trials contributes to deficits in performance. A similar analysis could equally well be applied to DRL performance (Tonkiss et al. 1988). With the caveat that perhaps we were unable to reach sufficiently high concentrations in the relevant brain areas, the present results suggest that AMPA receptor-mediated mechanisms do not play an essential role in such processes.

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