ELSEVIER

European Journal of Pharmacology 336 (1997) 143–154

ejp

Apparent antinociceptive and anti-inflammatory effects of GYKI 52466

József I. Székely *, Rita Kedves, Ildikó Máté, Katalin Török, István Tarnawa

Institute for Drug Research, P.O. Box 82, 1325 Budapest, Hungary

Received 3 April 1997; revised 5 August 1997; accepted 8 August 1997

Abstract

GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine) was examined in a battery of analgesia and anti-inflammatory tests in rats and mice, respectively. Its 3-*N*-acetyl (GYKI 53773) and 3-*N*-methylcarbamoyl (GYKI 53784) derivatives were also examined in some assays. These 2,3-benzodiazepines, known as prototypic non-competitive antagonists of AMPA receptors, showed a peculiar profile in some routinely used antinociceptive tests. They were found fairly potent in rat tail flick and mouse phenylquinone writhing assays but the dose–response curves were rather shallow as compared to that of morphine. Their action is stereoselective, i.e., the (+) isomers were found inactive, in agreement with the previous in vitro studies. Their antinociceptive effect could not be reversed by naloxone and the GYKI compounds did not potentiate significantly the morphine-induced analgesia. In the mouse hot plate assay the 2,3-benzodiazepines were active only in doses inducing visible motor incapacitation. In rats, GYKI 52466 weakly reduced the hypersensitivity accompanying acute carrageenan edema. However, it potently inhibited the hyperalgesia during Freund adjuvant-induced chronic arthritis. In the latter assay GYKI 52466 also attenuated the body weight loss without altering the paw edema. The present findings confirm reports in the literature which indicate AMPA receptors may contribute to certain forms of pathological hyperalgesia, e.g., to that detectable in inflamed tissues. © 1997 Elsevier Science B.V.

Keywords: GYKI 52466; GYKI 53773; GYKI 53784; 2,3-Benzodiazepine; Analgesia; Anti-inflammatory drug

1. Introduction

GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine) is the prototype of a novel class of drugs with a unique pharmacological profile as demonstrated in our laboratory (Tarnawa et al., 1989). Of the 2,3-benzodiazepines developed earlier, also termed 'homophtalazines', tofisopam is still used clinically as a non-sedative anxiolytic agent free of muscle relaxant side-effects. However, GYKI 52466, contrary to other 2,3-benzodiazepines, does possess marked muscle relaxant and anticonvulsant activities (Tarnawa et al., 1989). The main distinctive structural feature of GYKI 52466 is the 7,8-methylenedioxy bridge over the 2,3-benzodiazepine nucleus not present in tofisopam and other 2,3-benzodiazepines characterized earlier. GYKI 52466 is not a prototypical ligand of 'classical' (diazepam-like) 1,4-benzodiazepine receptors. Namely it displaces labeled diazepam only in the micromolar concentration range (Tarnawa et al., 1989) and does not compete with [^{3}H]flumazenil (De Sarro et al., 1995). Furthermore, it does not bind to the distinct binding sites of tofisopam described at our institute (Horváth et al., 1993).

The unique pharmacological character of GYKI 52466 was revealed by examination of the afferent nerve stimulation-induced ventral root potentials in spinal cats. GYKI 52466 reduces both the mono- and polysynaptic reflex responses, unlike midazolam, which only inhibits the latter (Tarnawa et al., 1989). Flumazenil, a selective antagonist of 1,4-benzodiazepine receptors, does not reverse the GYKI 52466-induced inhibition of flexor reflexes (Farkas et al., 1992). It is now well established that GYKI 52466 is a highly selective but non-competitive antagonist of AMPA/kainate receptors acting on a novel modulatory site coupled allosterically to them (Donevan and Rogawski, 1993).

GYKI 52466 and some of its 3-*N*-substituted analogs in mice selectively block the convulsions induced by AMPA ((*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid) and kainate but not by NMDA (*N*-methyl-D-aspartic acid) (Smith and Meldrum, 1990; Donevan et al., 1994). Furthermore, sound-induced convulsions in DBA/2 mice can be reversed by GYKI 52466

* Corresponding author. Tel.: (36-1) 169-0011; Fax: (36-1) 169-3229.

similarly to NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline), a competitive antagonist of AMPA receptors (Chapman et al., 1993). In this latter study, aniracetam, a positive modulator of AMPA receptors, antagonized the anticonvulsant actions of both GYKI 52466 and NBQX (Chapman et al., 1993). In cortical slices cyclothiazide, another AMPA positive modulator, also reversed the AMPA receptor antagonist action of GYKI 53655, the racemic form of GYKI 53784 (Palmer and Lodge, 1993).

Budai and Larson (1994) tested the effect of GYKI 52466 on the activity of wide dynamic range dorsal horn neurons in rat spinal cord. In most cells the responses to iontophoretically applied kainate and AMPA were suppressed by GYKI 52466 but those elicited by NMDA were not. Furthermore the responses of the same neurons to both noxious and innocuous mechanical stimulation were suppressed by GYKI 52466 (Budai and Larson, 1994). These findings show that the above described 'GYKI-sites' might also contribute to the regulation of nociceptive reflexes.

Otherwise, an increasing body of data indicates that glutamate receptors and glutamatergic mechanisms are involved in nociceptive reflexes, i.e., pain transmission, primarily at the spinal level (Schouenborg and Sjölund, 1986; Davies and Lodge, 1987; Dickenson and Sullivan, 1990; Haley et al., 1990; Song and Zhao, 1993). Analgesia is one of the potential uses for the glutamate antagonists currently under development (for review, see Danysz et al., 1995; Dray and Urban, 1996).

However, to our knowledge, discounting our preliminary reports (Székely et al., 1995, 1996), GYKI 52466 has not been tested for antinociceptive/anti-inflammatory effects in awake animals. For this reason we now examined the effects of GYKI 52466 in several routinely used antinociceptive and anti-inflammatory assays where the efficacy of clinically used opiate analgesics and prostaglandin biosynthesis inhibitors is well established. Where appropriate the actions of GYKI 52466 were compared to those of morphine, paracetamol and indomethacin and two potent analogs of the title compound were also examined in several assays.

2. Materials and methods

2.1. Animals

Experimental animals were purchased from Charles River. Generally, male mice of the CD1 strain (originating from the Institute for Cancer Research) (weighing 20–25 g) were used but the hot plate test was done with NMRI (Naval Medical Research Institute) mice. Male Lewis rats (weighing 120–160 g) were used for the adjuvant arthritis assay and otherwise SPRD (Sprague–Dawley) rats weighing 150–200 g were used. The animals were kept in the colony room for at least a week after arrival. The temperature was 20 ± 2°C, the humidity 40–60%. The lights were on from 7.00 a.m. to 7.00 p.m. Standard semi-synthetic food (also purchased from Charles River) and tap water were available ad libitum but in acute studies the orally treated animals were deprived of food for 18–20 h prior to the experiment. The study was carried out according to the guidelines of the Institutional Committee for Animal Welfare.

2.2. Procedures

2.2.1. Writhing assays in mice

Writhing was induced by intraperitoneal (i.p.) injection of freshly prepared 3.0% acetic acid or 0.1% phenylquinone solution. The irritants were injected in a volume of 0.1 ml/10 g body weight (Hendershot and Forsaith, 1959; Witkin et al., 1961). Immediately after i.p. administration of the irritant the animals were placed individually in Perspex boxes (8 × 8 × 6.5 cm) and observed for 20 min; 6 animals were monitored simultaneously by an observer unaware of the pretreatment. The distinct writhing movements of each animal were recorded separately. On each experimental day 3–5 groups ($n = 6$) were examined, one of them pretreated with vehicle. When the results for various vehicle-pretreated groups, examined on different experimental days, were pooled the mean number of writhes/group ± S.D. were 233 ± 21 and 250 ± 50 after i.p. application of acetic acid and phenylquinone solution, respectively. The drug-induced inhibition of writhing was expressed in per cent of the number for the control group examined on the same day, with

$$\%\text{inhibition} = 100 - \left(\frac{\text{drug pretreated}}{\text{vehicle pretreated}} \times 100 \right)$$

2,3-benzodiazepines were administered orally 15 min and morphine s.c. 30 min prior to i.p. injection of the irritant. In a separate series of experiments the animals were pretreated orally with 100 mg/kg aniracetam 30 min prior to administration of GYKI 52466.

The compounds were examined at 3–4 dose levels and the ED_{50} values were calculated according to Litchfield and Wilcoxon (1949). The slope function (S_f) of the dose–response curves was calculated, also according to Litchfield and Wilcoxon (1949) where

$$S_f = \frac{ED_{84}/ED_{50} + ED_{50}/ED_{16}}{2}$$

With these statistics S_f will be small (close to unity) if the dose–response curve is steep and a high S_f means a flat dose–response relationship (unlike in the case of regular calculation of regression slopes S_r, see Section 2.2.2).

2.2.2. Tail flick test in rats

The method of D'Amour and Smith (1941) was employed. The apparatus was constructed at our institute. A high intensity beam of light was focused on the tail around

the boundary of the middle and distal third. Reflex removal of the tail exposed a photocell to the light source and stopped the timer measuring the latency to the nearest 0.1 s. The intensity of the light generated by a projection bulb was regulated so that the control latency time (B) varied between 2.5 and 4.5 s. (Animals showing shorter or longer baseline latency were discarded.) The cutoff time was 15 s. The reading was repeated 15, 30, 45 and 60 min after drug treatment. (In case of GYKI 52466 the first reading was made at 5 min but the last reading was omitted in view of the relatively fast start and short duration of action.) The effects of morphine and 2,3-benzodiazepines were examined by administering the drugs to separate groups of animals ($n = 10–20$) at 3–4 logarithmically spaced dose levels.

The interaction of 2,3-benzodiazepines with morphine and naloxone was tested in another series of experiments. In one study, immediately prior to s.c. injection of morphine separate groups of rats ($n = 10$) were pretreated with either saline or one of the 2,3-benzodiazepines in doses close to their ED_{50} values when given alone. These doses of the 2,3-benzodiazepines were combined with increasing doses of morphine. Thus the ED_{50} value of morphine could be determined in the presence and absence of the 2,3-benzodiazepines. In another experiment, 10 mg/kg naloxone was injected s.c. to various groups of rats ($n = 10$) immediately after oral administration of one of the GYKI compounds in doses close to twice their ED_{50} values to see whether their antinociceptive action is reversible by an opiate antagonist.

For data analysis the 'mean possible effect' (%MPE) was first calculated according to the formula

$$\%\text{MPE} = \frac{\text{post-treatment}_{\text{latency}} - \text{baseline}_{\text{latency}}}{\text{cutoff time} - \text{baseline}_{\text{latency}}} \times 100$$

The doses of the drug under study were plotted against the %MPE and the data were analyzed by least square regression analysis. The ED_{50} values ($\pm 95\%$ confidence intervals) and the slopes of the regression lines (S_r) were calculated according to Bolton (1990). Of course high S_r values indicate steep dose–response curves and low S_r means flat dose–response relationship whereas the meaning of high and low S_f values is the opposite. Obviously S_f and S_r are related to each other in a roughly reciprocal fashion. Nevertheless if the biological responses are measured quantally S_f is more reliable descriptor of dose–effect relationships than S_r, i.e., the slope of the linear regression lines.

2.2.3. Hot plate test in mice

The test was performed according to Eddy et al. (1950) with minor modifications. An 'analgesimeter' from the Columbus Institute (Columbus, OH, USA) was used. The mice were dropped on a metal plate heated to 56 ± 0.5°C within a glass enclosure and the latency of hind paw lick was measured to the nearest 0.1 s. The baseline latency (B) was redetermined within 15 min and averaged. If B exceeded 15 s or the difference between two successive control measurements was > 4 s, readings from this animal were discarded. After control measurements the animals received the drug and the latency time was redetermined 15, 30, 45, 60 or 75 min thereafter. The cutoff latency was set to 30 s to prevent tissue damage. %MPE was calculated as in the case of the tail flick test (see Section 2.2.2).

2.2.4. Examination of paw edema

Edema was induced by injecting either 0.1 ml of 1% carrageenan or 0.05 ml of 2% zymosan suspension into the plantar surface of the right hindpaw of rats or mice, respectively. Paw volume was determined by plethysmometry immediately prior to and every 30 min for 3 h after carrageenan, or hourly for 6 h after injection of zymosan into the right paw. (The paw was submerged into a mercury-filled cell and the volume displaced was read in mm.) The paw volume was expressed in units, where 1 unit (U) corresponds to 0.0357 ml and 0.013 ml in rats and mice, respectively. To quantitate paw edema, the control value for paw volume determined before injection of the irritant was subtracted from the post-treatment volumes. These differences (Δ volumes) were then used as a measure of inflammation.

2.2.5. Rat paw pressure (Randall–Selitto) test

Basically the procedure of Randall and Selitto (1957) was followed. Male SPRD rats (160–200 g) were used. Carrageenan (0.1 ml of 1% solution) was injected into the plantar surface of the right hindpaw. Then the mechanical pain threshold of the inflamed hindpaw was determined with an 'analgesimeter' (Ugo Basile, Comerio-Varese, Italy). The inflamed hindpaw was placed on a small plinth and a blunt wedge-shaped teflon piston was positioned on the convex surface of the paw. The pressure was progressively increased, i.e., the paw was compressed until the animal withdrew its leg. The threshold pressure eliciting vigorous withdrawal movements was expressed in grams. To adapt the rats to the procedure two 'dummy' measurements were made before carrageenan injection. Then the reading was repeated 180, 240 and 300 min after carrageenan treatment, i.e., when the irritant-induced inflammation was the most intense. During each reading the threshold was determined twice successively and the average was recorded. Drugs were given orally either immediately before intraplanar injection of carrageenan or 165 min later, i.e., 15 min prior to the first reading. In separate experiments the drug was applied locally, i.e., into the right hindpaw at the times indicated above.

2.2.6. Adjuvant arthritis in rats

The method of Newbould (1963) was used, supplemented with the determination of the mechanical pain

threshold of the inflamed foot (Stein et al., 1988). For measurement of pain sensitivity the Randall and Selitto (1957) assay was used as described above. Arthritis was induced by intraplantar injection of 0.1 ml Freund adjuvant (2.5 mg heat-inactivated *Mycobacterium butyricum* suspended in 1 ml paraffin oil) into the right hindpaw. GYKI 52466 or vehicle was administered orally daily up to day 21 after Freund adjuvant treatment. The volumes of the right and left hindpaws and the body weight were determined on days 0, 3, 6, 10, 13, 17, 20 and 22. The mechanical pain threshold of the adjuvant-treated paw was measured on days 0, 2, 9, 16 and 22. For statistical evaluation of paw edema and body weight changes, the differences (Δ) were first calculated individually by subtracting the control values determined on day 0 from those recorded subsequently.

2.3. Drugs and chemicals

GYKI 52466 (1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine) and two of its potent dihydro-derivatives, including the 3-*N*-acetyl (GYKI 53773) and the 3-*N*-methylcarbamoyl (GYKI 53784) congeners, were synthesized at our institute by Dr. T. Hámori, Dr. I. Ling and Dr. G. Somogyi as described earlier (Tarnawa et al., 1993). Morphine (Alkaloida, Hungary), paracetamol (Chinoin, Hungary) and indomethacin (Sigma), phenylquinone (Sigma), acetic acid (Erdőkémia, Hungary), carrageenan (Roth, Germany), zymosan (Human, Hungary) and *Mycobacterium butyricum* (Difco) were obtained from commercial sources. The 2,3-benzodiazepines, indomethacin and paracetamol, were suspended in a drop of Tween-80 then diluted with saline. The other compounds used were soluble in saline. Indomethacin and paracetamol were given orally and morphine, s.c., the irritants intracutaneously into the right hindpaw or i.p. as specified above and GYKI 52466 in one experiment, locally. Otherwise the 2,3-benzodiazepines were administered orally and the volumes given were 0.5 ml/100 g and 0.1 ml/10 g body weight for rats and mice, respectively.

2.4. Statistics

Means ± S.E.M. were calculated and the drug effects were analyzed as specified above. Statistical significance was assessed by one-way or two-way analysis of variance (ANOVA) followed by the Newman–Keuls assay for multiple comparisons. For computations on a PC the following software was used: CSS/StatSoft and Excel (5.0), Microsoft; SAS System (6.1), SAS Institute.

3. Results

3.1. Effect in mouse writhing assays

As shown in Table 1, in the phenylquinone writhing assay the ED_{50} values for s.c. morphine and oral paracetamol were 0.54 and 160 mg/kg, respectively, in accordance with data in the literature. The relative potency of GYKI 52466 (ED_{50} = 4.96 mg/kg orally) was about an order of magnitude less than that of morphine whereas GYKI 53784 and GYKI 53773 (with ED_{50} values of 0.51 and 0.13 mg/kg) were found approximately equiactive to morphine and about four times more potent, respectively. The (+) isomers of GYKI 53773 and GYKI 53784 were inactive up to 30.0 mg/kg. Thus the rank order of potencies was:

GYKI 53773 > GYKI 53784
~ morphine > GYKI 52466 ≫ paracetamol.

However, the dose–response curves for GYKI 52466, GYKI 53784 and GYKI 53773 were more shallow than those for morphine and paracetamol (Table 1). Namely, the slope functions (the S_f values) were significantly higher ($P < 0.05$) for the GYKI compounds than for morphine (see Table 1).

Table 1
Effects of 2,3-benzodiazepines, morphine and paracetamol in phenylquinone writhing assay in mice

Compound examined [a] (stereospecificity)	ED_{50} [b] (mg/kg p.o.) (±95% conf. int.)	Slope function [c] (S_f)	Relative potency
Morphine	0.54 (s.c.) (0.38–0.77)	1.86	1
Paracetamol	160 (83.3–307)	2.26	0.003
GYKI 52466	4.96 (2.5–9.88)	5.59	0.11
GYKI 53773 (−)	0.13 (0.02–0.70)	13.3	4.19
GYKI 53774 (+)	> 30 [d]	—	—
GYKI 53784 (−)	0.51 (0.08–3.07)	15.6	1.06
GYKI 53785 (+)	> 30 [d]	—	—
Aniracetam [e] + GYKI 52466	9.25 (4.9–17.6)	4.02	0.06

[a] All compounds were administered orally 30 min prior to i.p. injection of phenylquinone but morphine was injected s.c. 15 min prior to the irritant.
[b] From % inhibition of writhing, ED_{50} ± 95% confidence interval was calculated according to Litchfield and Wilcoxon (1949). Compounds were examined at 3–4 logarithmically spaced dose levels (n = 6–12/dose).
[c] Slope function (S_f) was calculated according to Litchfield and Wilcoxon (1949).
[d] Inhibition < 50% at the dose indicated.
[e] 100 mg/kg aniracetam was given orally 30 min prior to various doses of GYKI 52466.

In the acetic acid writhing assay the ED_{50} of GYKI 52466 was 51.8 mg/kg (95% conf. int.: 16.4–163 mg/kg). Thus, GYKI 52466 was about an order of magnitude less potent in the acetic acid than in the phenylquinone writhing assay contrary to morphine (s.c. ED_{50} = 0.25 mg/kg; 95% conf. int.: 0.12–0.52 mg/kg) which was found nearly equiactive regardless of which irritant was used. In this assay also the slope factor (S_f) for the dose–response curve of GYKI 52466 (17.7) was higher, i.e., the dose–response curve was much more shallow ($P < 0.05$) than that for morphine (4.78) (see Section 2.2.1 for interpretation of S_f).

The effect of pretreatment with aniracetam was examined in a separate experiment. The ED_{50} value of GYKI 52466 in the phenylquinone writhing assay in the presence of aniracetam (100.0 mg/kg given orally prior to GYKI 52466) was 9.25 mg/kg. This value was about twice that without aniracetam but the difference was not statistically significant (Table 1).

3.2. Effect in rat tail flick test

As shown in Fig. 1, morphine and the 2,3-benzodiazepines elicited a dose-related increase in tail flick latency. At the highest doses examined (3.0 mg/kg of morphine s.c. or 3.0 mg/kg of GYKI 53773 and GYKI 53784 and 30.0 mg/kg of GYKI 52466, all given orally) the drugs induced near maximal lengthening of the latency time, close to the cutoff value (15 s). The effect of s.c. morphine peaked at 15–30 min, whereas the duration of

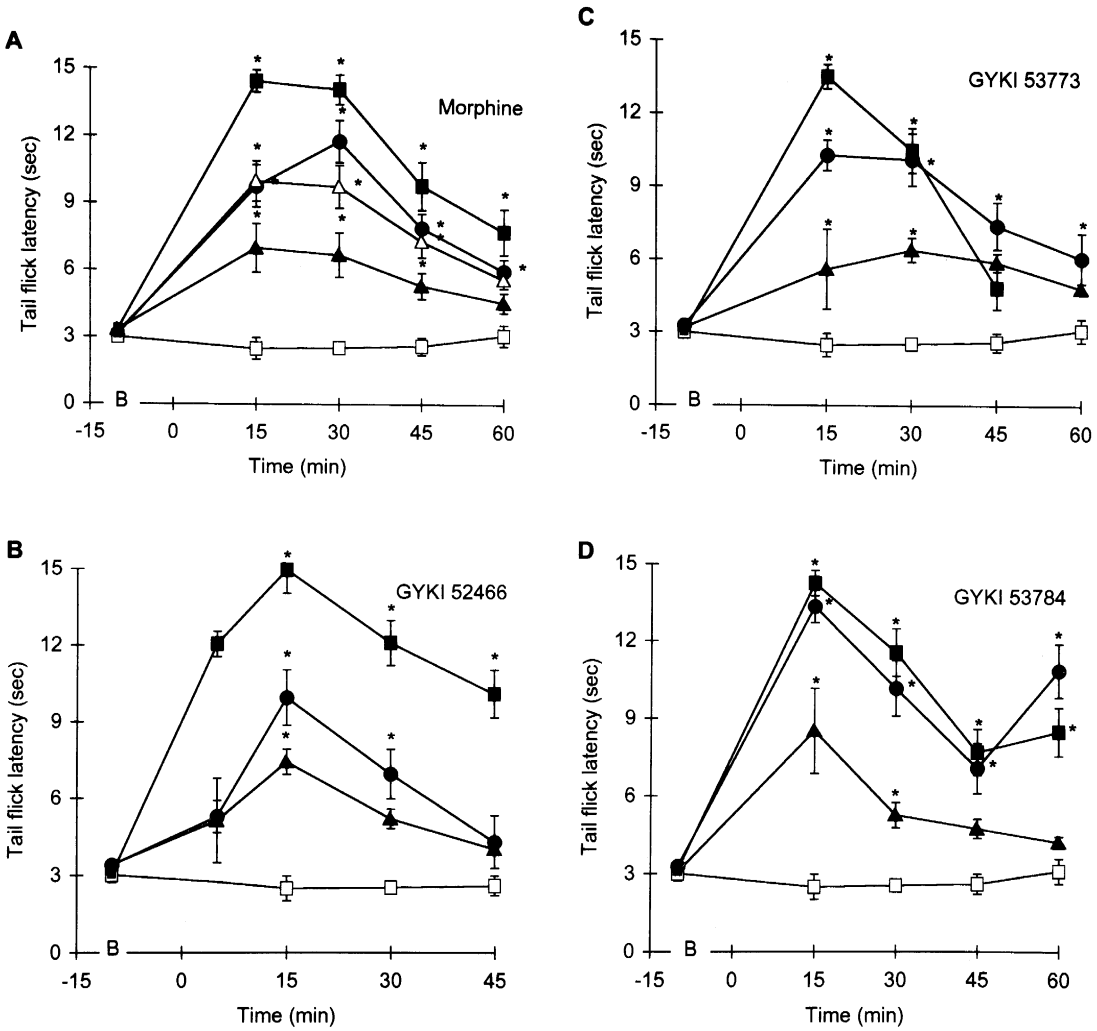

Fig. 1. Time-course and dose dependence of the increase in tail flick latency (ordinate) induced by morphine (A), GYKI 52466 (B), GYKI 53773 (C) and GYKI 53784 (D). On the abscissa, the time elapsed after treatment. B = baseline latency. Morphine was administered s.c., the GYKI compounds per os. Each point corresponds to the mean ± S.E. for 10–20 rats. * $P < 0.05$ comparing the drug-treated groups to the saline-treated ones in the same experiment at the corresponding time point (ANOVA followed by Newman–Keuls assay). To avoid crowding of the figure, the 10 mg/kg dose of GYKI 52466, the 1 mg/kg dose of all the GYKI compounds and the 0.1 mg/kg dose of GYKI 53773 and GYKI 53784 have been omitted but are shown in Fig. 2. (-□-) saline on each panel. In panel A (-▲-), (-△-), (-●-) and (-■-) correspond to 0.1, 0.3, 1.0 and 3.0 mg/kg morphine. In panel B (-△-), (-●-) and (-■-) denote 0.3, 3.0 and 30.0 mg/kg of GYKI 52466. In panels C and D (-△-), (-●-) and (-■-) indicate 0.003, 0.3 and 3.0 mg/kg of GYKI 53773 and GYKI 53784, respectively.

Table 2
Effects of 2,3-benzodiazepines, morphine and paracetamol in tail flick assay in rats

Compound examined [a] (stereospecificity)	ED_{50} [b] (mg/kg p.o.) (±95% conf. int.)	Slope of the regression line [c] (S_r)	Relative potency
Morphine	0.27 (s.c.) (0.15–0.42)	18.2	1
Paracetamol	> 600 [d]	—	—
GYKI 52466	1.38 (0.46–2.85)	13.1	0.19
GYKI 53773 (−)	0.11 (0.06–0.22)	11.1	2.35
GYKI 53774 (+)	> 30 [d]	—	—
GYKI 53784 (−)	0.02 (0.006–0.06)	8.32	11.2
GYKI 53785 (+)	> 30 [d]	—	—

[a] All compounds were administered orally, but morphine was injected s.c.
[b] ED_{50} ± 95% confidence interval was calculated from mean percent analgesia at time of peak effect, by least-squares linear regression of the individual data (Bolton, 1990). Compounds were examined at 3–4 logarithmically spaced dose levels ($n = 10–20$/dose).
[c] Slope of the regression line (S_r) was calculated by least-squares linear regression (Bolton, 1990).
[d] Inhibition < 50% at dose indicated.

action of GYKI compounds was shorter, with a peak effect at 15 min (Fig. 1).

In this test the ED_{50} values of morphine (s.c.) and orally given GYKI 52466, GYKI 53773 and GYKI 53784 were 0.27, 1.38, 0.11 and 0.02 mg/kg, respectively (Table 2). (Paracetamol was inactive in this assay.) Thus, in this test, GYKI 53784 was somewhat more potent than GYKI 53773 but the rank order of potencies was otherwise similar to that seen in the phenylquinone writhing assay:

GYKI 53784 > GYKI 53773 > morphine > GYKI 52466.

The slopes of the regression lines, i.e., the S_r values, were 18.2, 13.1, 11.1 and 8.32 for morphine, GYKI 52466, GYKI 53773 and GYKI 53784, respectively (Table 2 and

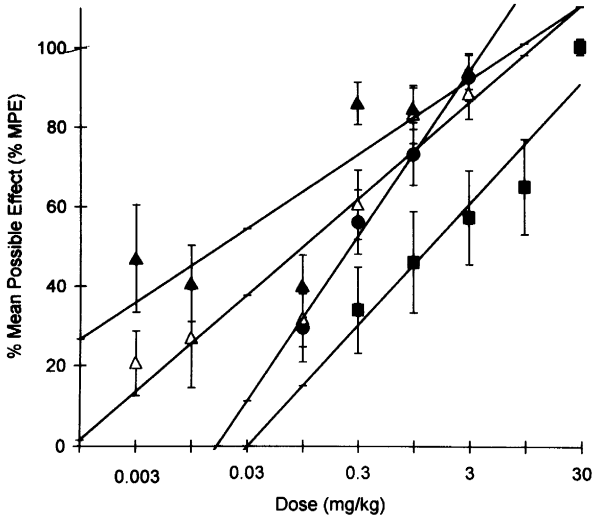

Fig. 2. Dose–response relationships of s.c. injected morphine and orally administered 2,3-benzodiazepines in rat tail-flick assay. On the ordinate the mean percent analgesia calculated as described in Section 2.2.2 and on the abscissa the doses on a logarithmic scale. Mean possible effect was calculated from the latency times read at 15 min (GYKI compounds) or 30 min (morphine) after treatment i.e. at the time of maximal analgesia. The values are means ± S.E. for 10–20 rats. The linear regression lines (for numerical values see Table 2) were calculated according to Bolton (1990). Symbols (-●-), (-■-), (-△-), (-▲-) represent various doses of morphine, GYKI 52466, GYKI 53773 and GYKI 53784, respectively.

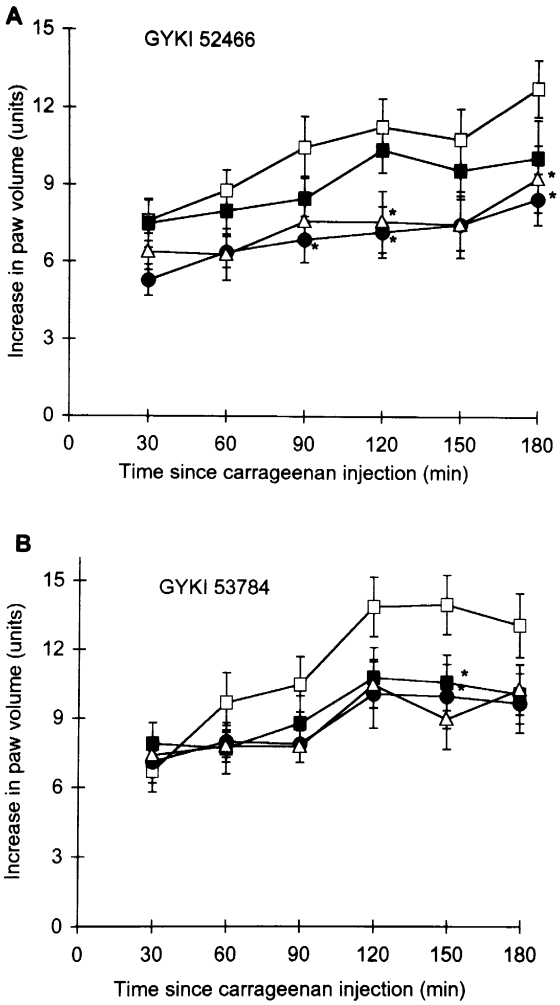

Fig. 3. The effects of GYKI 52466 (10, 30 and 100 mg/kg per os) (A) and GYKI 53784 (1, 3 and 10 mg/kg per os) (B) on carrageenan-induced paw edema in rats. The paw volume was determined by plethysmometry immediately prior to, and every 30 min for 3 h, after intraplantar injection of 0.1 ml 1% carrageenan suspension. The carrageenan-induced increase in paw volume is expressed in units (1 unit = 0.0357 ml). The values are means ± S.E. of 12 rats. * $P < 0.05$ drug versus vehicle-treated group (ANOVA followed by Newman–Keuls assay). Symbols (-□-), (-■-), (-●-) and (-△-) correspond to saline and 10.0, 30.0 and 100.0 mg/kg GYKI 52466 in panel A and 1.0, 3.0 and 10.0 mg/kg GYKI 53784 in panel B.

Table 3
Modulation by 2,3-benzodiazepines of morphine analgesia in rat tail flick test

Pretreatment (mg/kg p.o.)	Treatment (s.c.)	ED_{50} (mg/kg s.c.) (±95% conf. int.)
Saline	morphine	0.27 (0.15–0.42)
GYKI 52466, 1.0	morphine	0.07 (0.28–4.05)
GYKI 53773, 0.1	morphine	0.30 (0.09–0.98)
GYKI 53784, 0.01	morphine	0.25 (confidence interval could not be determined)

See Section 2.3 and Table 2 for calculation of ED_{50} ± 95% confidence interval. Morphine was examined at 3–4 logarithmically spaced dose levels ($n = 10–20$). Pretreatment orally with saline or the 2,3-benzodiazepine compound immediately prior to s.c. injection of morphine. Testing 15 min after treatment.

Fig. 2). Thus, in this assay also, the dose–response curves for 2,3-benzodiazepines were apparently less steep than that of morphine. However, according to so called 'parallel line bioassay' (Bolton, 1990) the difference in slopes was statistically significant only when morphine was compared to GYKI 53784.

This assay also evaluated the stereospecificity. GYKI 53774 and GYKI 53785, the (+) isomers of GYKI 53773 and GYKI 53784, were found inactive up to 30.0 mg/kg. (GYKI 52466 is not a chiral compound.) Thus, the effect of 2,3-benzodiazepines was stereospecific.

To examine a possible interaction of 2,3-benzodiazepines with morphine, separate groups of rats were pretreated orally with 1.0 mg/kg of GYKI 52466, 0.1 mg/kg of GYKI 53773 or 0.01 mg/kg of GYKI 53784 before the s.c. injection of increasing doses of morphine. As shown in Table 3, GYKI 53773 and GYKI 53784 did not modify significantly the ED_{50} of morphine. GYKI 52466 induced a four-fold reduction of ED_{50} (from 0.27 to 0.07 mg/kg) but the confidence intervals overlapped. Thus 2,3-benzodiazepines neither considerably potentiated nor inhibited the effect of morphine in this assay, though some weaker degree of interaction cannot be excluded based on this experiment (Table 3).

In another experiment naloxone was injected s.c. to various groups of rats immediately after oral administration of the GYKI compounds in doses close to twice their analgesic ED_{50} values. In this experiment the doses of GYKI 52466, GYKI 53773 and GYKI 53784 were 2.0, 0.2 and 0.02 mg/kg, respectively. Naloxone, 10.0 mg/kg s.c. failed to inhibit significantly the antinociceptive action of these 2,3-benzodiazepine derivatives: they elicited analgesia above 60% MPE even after pretreatment with this high dose (data not shown). Thus the antinociceptive effect of 2,3-benzodiazepines seems to be basically resistant to antagonism by naloxone, though slight alterations in their efficacies cannot be excluded based on the combination of single doses.

3.3. Effect in mouse hot plate test

In this test, contrary to the writhing and tail flick assays, GYKI 52466 was almost inactive (the ED_{50} of morphine was 7.6 mg/kg). GYKI 52466 induced significant prolongation of the paw-lick reaction time only with the 100.0 mg/kg (oral) dose with MPE of 76%, 73%, 69% and 58% at the 15, 30, 45 and 60 min readings, respectively. However, as reported earlier (Tarnawa et al., 1989) at this high dose level this compound also induces motor impairment, muscle relaxation and ataxia. GYKI 53773 and GYKI 53784 were tested only in a pilot experiment. At 10 mg/kg oral doses both failed to induce antinociception exceeding 50% MPE.

3.4. Effects in acute models of inflammation and hyperalgesia

3.4.1. Carrageenan-induced paw edema in rats

The edema was moderately but statistically significantly reduced by 30.0–100.0 mg/kg of GYKI 52466 or 3.0–10.0

Table 4
Effect of GYKI 52466 on mechanical pain threshold of inflamed paw (Randall–Selitto test)

Treatment (mg/kg p.o.) 165 min after carrageenan	Mechanical pain threshold [a], mean ± S.E. (in g)			
	180 min [b]	240 min [b]	300 min [b]	180–300 [c]
Saline	6.8 ± 0.4	8.7 ± 1.2	9.7 ± 1.2	8.4 ± 0.8
GYKI 52466, 10.0	9.0 ± 0.8	8.0 ± 1.0	11.2 ± 2.9	9.4 ± 0.9
GYKI 52466, 30.0	8.3 ± 0.6	11.3 ± 2.1	11.1 ± 1.6	10.2 ± 0.9
GYKI 52466, 100.0	33.9 [×] ± 10.4	17.8 [×] ± 4.6	13.4 ± 2.4	21.7 ± 6.2
Saline locally into the inflamed paw	12.9 ± 1.3	18.8 ± 3.5	17.7 ± 3.3	16.5 ± 1.8
GYKI 52466 1.0 mg locally into the inflamed paw	28.0 ± 4.5	28.1 ± 6.1	29.4 ± 6.8	28.5 ± 0.5

[×] $P < 0.05$: ANOVA followed by Newman–Keuls assay ($n = 10$/dose).
[a] 0.1 ml of carrageenan (1%) was injected into the right hindpaw to induce inflammation and hyperalgesia.
[b] Time elapsed since carrageenan injection into the right paw.
[c] Overall mean of the data read at 180, 240 and 300 min.

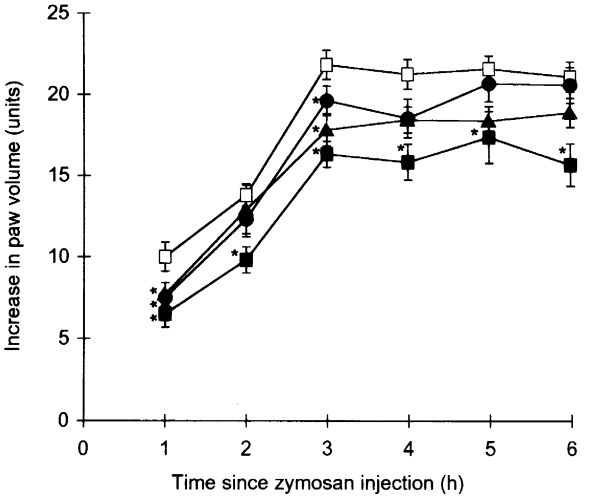

Fig. 4. The effects of 2,3-benzodiazepines on zymosan-induced paw edema in mice. Paw volume was determined by plethysmometry immediately prior to and 1, 2, 3, 4, 5 and 6 h after intraplantar injection of 0.05 ml 2% zymosan solution. All the drugs were given orally in a 10-mg/kg dose immediately after zymosan. Increase in paw volume is expressed in units (1 unit = 0.013 ml). The values are means ± S.E. for 10 mice. * $P < 0.05$ drug versus vehicle treated group. Symbols (-□-), (-●-), (-▲-) and (-■-) denote the groups pretreated with saline, GYKI 52466, GYKI 53773 and GYKI 53784, respectively.

mg/kg of GYKI 53784 (Fig. 3). This effect was maximal 2–3 h after carrageenan injection, similarly to the classical prostaglandin synthesis inhibitors. However, the maximal reduction in paw volume was 25–30% while it is well established that genuine anti-inflammatory agents, in appropriate doses, induce at least 50% reduction of carrageenan edema.

3.4.2. Zymosan-induced paw edema in mice

The edema was hardly modified by 10.0 mg/kg of GYKI 52466 but was inhibited to a statistically significant extent by the same doses of GYKI 53773 and GYKI 53784 (Fig. 4). No dose–response relation was tested in the zymosan edema assay. Therefore it can only be seen that, at relatively high dose levels, 2,3-benzodiazepines induce moderate but statistically significant inhibition, GYKI 53773 and GYKI 53784 apparently being more potent than the parent compound. However, all were found weaker than indomethacin had been in our earlier experiments (data not shown) and as reported in the literature.

3.4.3. Rat paw pressure (Randall–Selitto) test

Only GYKI 52466 was tested in this assay. Its oral application concomitantly with carrageenan failed to modify the pain threshold (data not shown). However, given 165 min after carrageenan injection, i.e., 15 min prior to the first reading, the 100.0 mg/kg dose significantly elevated the pain threshold (Table 4). GYKI 52466 also moderately raised the pain threshold if given locally, i.e., into the inflamed paw in a dose of 1.0 mg 165 min after carrageenan treatment (Table 4).

3.5. Effect on Freund adjuvant-induced chronic arthritis

GYKI 52466 was examined in this assay: 0.3–1.0–3.0 mg/kg of the compound was administered every day over the 3-week observation period. GYKI 52466 failed to antagonize significantly either the primary edema on the right paw or the secondary, generalized inflammation-induced swelling of the left paw developing in the second phase of the chronic disease (data not shown). However, it attenuated the inflammation induced hyperalgesia and body weight loss.

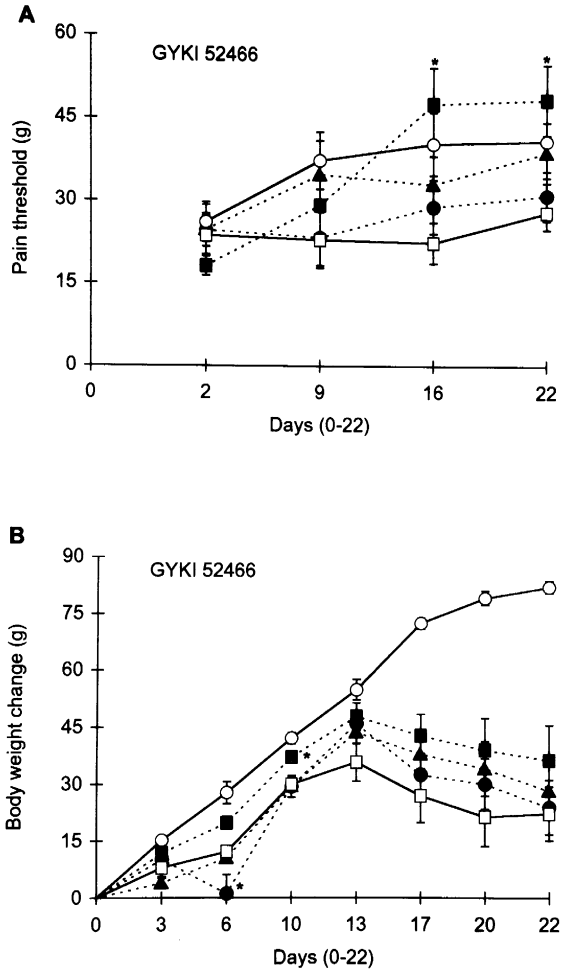

Fig. 5. Effects of GYKI 52466 on pain threshold (A) and body weight (B) during adjuvant arthritis in rats. The negative control group received saline orally and intraplantar vehicle. The positive controls received saline orally and intraplantar Freund adjuvant. The drug-treated groups received intra-paw Freund adjuvant and increasing doses of GYKI 52466 given daily, orally from day 1 to day 21. On the abscissa, days elapsed since induction of arthritis by Freund adjuvant (in both panels). On the ordinate, mechanical pain threshold (A) (determined according to Randall and Selitto, 1957) or the body weight change relative to day 0 (B). The values are means ± S.E. for 10 rats. * $P < 0.05$ drug versus positive control group. Separate groups of rats ($n = 12$) received Freund adjuvant + vehicle (-□-; positive controls) or Freund adjuvant + 0.3 (-●-) or 1.0 (-▲-) or 3.0 (-■-) mg/kg of GYKI 52466 orally from day 1 to day 22 or only vehicle (-○-; negative controls).

As shown in Fig. 5, significant hyperalgesia developed in the arthritic animals (compare the negative and positive controls on panel A). The arthritis-induced hyperalgesia was not completely abolished but significantly attenuated by GYKI 52466 (Fig. 5A). According to two-way ANOVA both the overall treatment and time effects were statistically significant ($F(4,180) = 4.75$; $P < 0.01$ and $F(3,180) = 7.77$; $P < 0.001$). A significant overall treatment effect was also found if the positive control and various treatment groups were analyzed separately by one-way ANOVA ($F(3,156) = 4.23$; $P < 0.01$). Comparing the various treatment levels in the Newman–Keuls test, the pain threshold of Freund adjuvant-treated positive controls was significantly lower than that of the untreated negative controls and this action was reversed by the highest, i.e., 3.0 mg/kg dose of GYKI 52466. When the experimental days were analyzed separately the effect of 3.0 mg/kg GYKI 52466 was statistically significant on days 16 and 22.

The relative weight loss was not eliminated but also significantly attenuated by the highest (3.0 mg/kg) dose of GYKI 52466 (Fig. 5B) (the overall treatment and time effects in the first phase of inflammation (days 3–10) and the treatment effect in the second one (days 13–22) were statistically significant, with $F(4,135) = 9.12$; $P < 0.001$, $F(2,135) = 160.67$; $P < 0.001$ and $F(4,180) = 31.38$; $P < 0.001$, respectively. Comparison of the positive control groups with the drug-treated ones by one-way ANOVA showed a significant overall treatment effect in both the first and second phases of inflammation $F(3,116) = 3.21$; $P < 0.05$ and $F(3,156) = 3.58$; $P < 0.05$, respectively). According to the paired comparisons the overall effects of 0.3 and 3.0 mg/kg doses were significant.

Observed subjectively, the drug-treated animals seemed to be less lethargic or immobile. They were moving about in the home cage more freely than the positive controls.

4. Discussion

The experimental data indicate that GYKI 52466 and two analogs (GYKI 53773 and GYKI 53784) had a potent antinociceptive action in the rat tail-flick and mouse phenylquinone writhing assays but were nearly inactive in the mouse hot plate test.

The efficacy in the first two assays does not seem to be secondary to muscle relaxation and/or motor impairment since these symptoms appeared at considerably higher dose levels. For example, Donevan et al. (1994) found the i.p. ED_{50} values of GYKI 52466 in maximal electroshock seizure (MES), kainate seizure and motor toxicity (i.e., horizontal/inclined screen) tests to be 11.8, 8.4 and 13.2 mg/kg, respectively. Earlier in our laboratory, the oral ED_{50} values of GYKI 52466 against MES, metrazole-induced convulsions and the muscle relaxant activity in the inclined screen test were found to be 38.0, 115.0 and 47.0 mg/kg, respectively (Tarnawa et al., 1993). However, in the present study the oral antinociceptive ED_{50} values of GYKI 52466 were 1.38 and 4.96 mg/kg in the rat tail flick and mouse phenylquinone writhing tests, respectively. The difference between antinociceptive and motor impairing doses was even more conspicuous in the case of GYKI 53773 and GYKI 53784. Namely, the apparent antinociceptive ED_{50} values of these compounds were well below 1 mg/kg per os (see Tables 1 and 2) whereas Donevan et al. (1994) found that the anticonvulsant and motor impairing doses of the racemic form of GYKI 53773 and GYKI 53784 (coded as GYKI 53405 and GYKI 53655) ranged between 5 and 10 mg/kg i.p. The flat slope of the dose–response curves warrants extreme caution in the interpretation of these surprising data.

As shown in Table 3, 2,3-benzodiazepines displayed no significant synergism with morphine. This finding is in line with the data of Advokat et al. (1994) on the lack of effect of intrathecal AMPA on morphine analgesia and those of Fundytus and Coderre (1994) on the inability of GYKI 52466 to suppress withdrawal symptoms in morphine-dependent mice. Thus, the antinociceptive action of these GYKI compounds does not seem to be related to opioid mechanisms.

Aniracetam, a selective agonist of AMPA receptors, in 100 mg/kg oral dose doubled the antinociceptive ED_{50} of GYKI 52466 in the writhing assay, but its inhibitory action was not statistically significant. However, De Sarro et al. (1995) could reverse the anticonvulsant action of GYKI 52466 when they i.c.v. administered aniracetam. Thus, it cannot be excluded that the dose of aniracetam applied by us was too small or should have been given centrally. Obviously further experiments are needed to examine the possible reversal by positive AMPA receptor modulators of GYKI 52466-induced antinociception.

2,3-benzodiazepines also showed anti-inflammatory actions in some acute models of inflammation, such as carrageenan- and zymosan-induced edema (Figs. 3 and 4). However, the effect of GYKI 52466 was statistically significant only at 100 mg/kg and the analogs were active at 10 mg/kg. These doses are about two orders of magnitude higher than their ED_{50} values for the tail-flick and writhing tests. On the other hand muscle relaxation and motor impairment, conspicuous at these doses, probably do not interfere with the irritant-induced swelling of the paw. Furthermore, 100 mg/kg oral and 1 mg local doses of GYKI 52466 reduced the mechanical hyperalgesia during carrageenan-induced edema (Table 4).

In the chronic arthritis assay, repeated small doses of GYKI 52466 failed to suppress either the primary or the secondary edema on the Freund adjuvant-treated and the contralateral sides, respectively. However, GYKI 52466 significantly elevated the pain threshold of the inflamed foot and blunted the progressively developing body weight loss (Fig. 5). The relative weight gain may also be related to elevation of the pain threshold. It is generally accepted that weight loss during adjuvant arthritis is due to tender-

ness of the inflamed paws, i.e., the rats might consume less food since they have to walk or stand on the sore legs to reach the feeder.

However, unexpected were these findings they seem to be compatible with other in vivo data about the mode of action of AMPA receptor antagonists.

As for the antinociceptive action of GYKI compounds, simultaneously with our preliminary publications (Székely et al., 1995, 1996), Kallman et al. (1995) reported (also in preliminary form) on the inhibition of acetic acid-induced writhing and lengthening of the tail-flick reaction time in mice on oral administration of 10 mg/kg LY-300164, which is the chemical code of Eli Lilly for GYKI 53773 (ED_{50} values were not specified in their study).

The apparent antinociceptive action of 2,3-benzodiazepines in behavioral experiments is consistent with several electrophysiological findings. In the spinal cord of urethane-anesthetized rats iontophoretic application of GYKI 52466 inhibits the firing of wide dynamic range dorsal horn neurons in response to both noxious and innocuous stimulation (Budai and Larson, 1994). The AMPA- and kainic acid-induced discharges of the same units are also inhibited by GYKI 52466 but not those elicited by NMDA (Budai and Larson, 1994). In a related study, systemic application of both NBQX and the racemic form of GYKI 53784 (coded as GYKI 53655) suppressed the noxious heat- and innocuous tap-induced discharges of wide dynamic range neurons in spinal rats (Cumberbatch et al., 1994). Recording of superficial dorsal horn neurons in a hamster lumbosacral slice preparation showed that the AMPA receptor antagonist, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), effectively suppressed the excitatory postsynaptic potentials induced by A- and C-fiber afferent stimulation whereas CPP ((3-(±)-2-carboxypiperazin-4-yl)-propyl-l-phosphonic acid), an NMDA receptor antagonist, only suppressed the late components (Näsström et al., 1992). Thus, in these experiments competitive antagonists of AMPA receptors, including NBQX and CNQX and the noncompetitive 2,3-benzodiazepines, showed similar actions. Recently Farkas and Ono (1995) demonstrated that i.v. administration of GYKI 52466, similarly to NBQX, powerfully suppressed the mono-, di- and polysynaptic ventral root reflex potentials in C_1 spinal rats. In the same study CPP and MK-801, i.e., prototypical competitive and non-competitive NMDA receptor antagonists were found to be only weak inhibitors of the mono- and polysynaptic ventral root reflexes (Farkas and Ono, 1995). Since intrathecal administration of excitatory amino acid agonists like NMDA, AMPA, quisqualate and kainic acid induces hyperalgesia and behavior (scratching and biting) indicating aversion (Aanonsen and Wilcox, 1987; Ferreira and Lorenzetti, 1994), it was proposed that various subtypes of glutamate receptors are involved in pain transmission. In a frequently cited study of Näsström et al. (1992) CNQX and DNQX (6,7-dinitro-quinoxalinedione) showed potent antinociceptive actions in the hot plate, tail-flick and formalin assays as well. However, of various NMDA receptor antagonists, only competitive inhibitors, e.g., 2-amino-5-phosphonopentanoic acid (AP-5) were active in the hot plate and formalin tests but not in the tail-flick assay and MK-801 was found completely inactive (Näsström et al., 1992). In a related study of Advokat and Rutherford (1995) intrathecally administered CNQX, in contrast to AP-5, was found active in the tail-flick assay in spinal and intact rats.

Nevertheless, if possible clinical application of glutamate antagonists is the context, certain forms of acute and chronic pain are probably more reliable predictors than the routinely used simple analgesia assays. In electrophysiological terms, 'wind up' of the C-fiber stimulation-elicited neuronal responses underlies the hyperalgesia induced by persistent pain stimulation (Hylden et al., 1989; Dickenson and Sullivan, 1990; Woolf and Thompson, 1991; Dray et al., 1994). There is now considerable experimental evidence showing that an excessive liberation of excitatory amino acids, tachykinins or dynorphin brought about by increased expression of early genes in the spinal cord dorsal horn mediates the enhanced responsiveness to C-fiber stimulation during acute and chronic inflammation (Dubner and Ruda, 1992; Urban et al., 1994; Malmberg and Yaksh, 1995; Harris et al., 1996). Furthermore, upregulation of both NMDA- and AMPA-sensitive glutamate receptors has been observed during chronic pain induced by ligation of the sciatic nerve (Harris et al., 1996). Thus, the hyperalgesia in chronic pain models is probably more relevant than the acute pain tests.

In our present experiments GYKI 52466 significantly reduced the adjuvant arthritis- (and carrageenan)-induced hyperalgesia. These findings are consistent with those of Sluka and Westlund (1993) who recorded simultaneous attenuation of inflammation and hyperalgesia upon intrathecal administration of CNQX. In their study, not only was the pathologically elevated pain threshold restored to normal but a decrease of swelling and attenuation of temperature increase over the knee joint, treated locally with kaolin + carrageenan, were also recorded. Several studies showed MK-801 and several other NMDA receptor antagonists to also be fairly potent to suppress the experimental hyperalgesia induced by carrageenan (Eisenberg et al., 1994), mustard oil (Woolf and Thompson, 1991), Freund adjuvant (Ren et al., 1992), prostaglandine E_2 (Ferreira and Lorenzetti, 1994) or ligation of the sciatic nerve (Davar et al., 1991; Mao et al., 1992), whereas the effect of NBQX or CNQX was found rather weak (Ren et al., 1992; Ferreira and Lorenzetti, 1994). Others, however, also found NBQX (Xu et al., 1993; Hunter and Singh, 1994) and CNQX (Mao et al., 1992; Sluka et al., 1993) active in these models. Jackson et al. (1995) reported recently that the thermal hyperalgesia induced by intraplantar injection of carrageenan could be reversed by locally applied MK-801 and CNQX as well. Thus, for the time being, the proposal of Mao et al. (1992) should be

accepted, that both NMDA- and non-NMDA receptor-mediated glutamatergic mechanisms are involved in experimental hyperalgesia and these processes may be complementary.

It remains to be seen whether the apparent antinociceptive potential of various types of glutamate antagonists can be utilized clinically.

Acknowledgements

The authors thank Dr. Júlia Singer for her advice with statistical processing, Mrs. Éva Kärtsch and Ms. Beáta Fehér for their technical help and Mrs. Mária Molnár and Ms. Andrea Parti for typing the manuscript.

References

Aanonsen, L.M., Wilcox, G.L., 1987. Nociceptive action of excitatory amino acids in the mouse: Effects of spinally administered opioids, phencyclidine and sigma agonists. J. Pharmacol. Exp. Ther. 243, 9–19.

Advokat, C., Rutherford, D., 1995. Selective antinociceptive effect of excitatory amino acid antagonists in intact and acute spinal rats. Pharmacol. Biochem. Behav. 51, 855–860.

Advokat, C., Prejean, J., Bertman, L., 1994. Intrathecal coadministration of morphine and excitatory amino acid agonists produce differential effects on the tail-flick of intact and spinal rats. Brain Res. 641, 135–140.

Bolton, S., 1990. Linear regression and correlation. In: Bolton, S. (Ed.), Pharmaceutical Statistics. Dekker, New York, NY, pp. 210–234.

Budai, D., Larson, A.A., 1994. GYKI 52466 inhibits AMPA/kainate and peripheral mechanical sensory activity. NeuroReport 5, 881–884.

Chapman, A.G., Al-Zubaidy, Z., Meldrum, B.S., 1993. Aniracetam reverses the anticonvulsant action of NBQX and GYKI 52466 in DBA/2 mice. Eur. J. Pharmacol. 231, 301–303.

Cumberbatch, M.J., Chizh, B.A., Headley, P.M., 1994. AMPA receptors have an equal role in spinal nociceptive and non-nociceptive transmission. Neuroreport 5, 877–880.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Danysz, W., Parsons, C.G., Bresink, I., Quack, G., 1995. Glutamate in CNS disorders. Drug News Perspect. 8, 261–277.

Davar, G., Hama, A., Deykin, A., Vos, B., Maciewicz, R., 1991. MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. Brain Res. 553, 327–330.

Davies, S.N., Lodge, D., 1987. Evidence for involvement of *N*-methyl-aspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. Brain Res. 424, 402–406.

De Sarro, G., Chimirri, A., De Sarro, A., Gitto, R., Grasso, S., Giusti, P., Chapman, A.G., 1995. GYKI 52466 and related 2,3-benzodiazepines as anticonvulsant agents in DBA/2 mice. Eur. J. Pharmacol. 294, 411–422.

Dickenson, A.H., Sullivan, A.F., 1990. Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurons in the rat. Brain Res. 506, 31–39.

Donevan, S.D., Rogawski, M.A., 1993. GYKI 52466, a 2,3-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/kainate receptor responses. Neuron 10, 51–59.

Donevan, S.D., Yamaguchi, S.-I., Rogawski, M.A., 1994. Non-*N*-methyl-D-aspartate receptor antagonism by 3-*N*-substituted 2,3-benzodiazepines: Relationship to anticonvulsant activity. J. Pharmacol. Exp. Ther. 271, 25–29.

Dray, A., Urban, L., 1996. New pharmacological strategies for pain relief. Annu. Rev. Pharmacol. 36, 253–280.

Dray, A., Urban, L., Dickenson, A., 1994. Pharmacology of chronic pain. Trends Pharm. Sci. 15, 190–197.

Dubner, R., Ruda, M.A., 1992. Activity-dependent neuronal plasticity following tissue injury and inflammation. Trends Neurosci. 15, 96–103.

Eddy, N.B., Fuhrmeister-Touchberry, C., Lieberman, J.E., 1950. Synthetic analgesics. I. Methadone isomers and derivatives. J. Pharmacol. Exp. Ther. 98, 121–137.

Eisenberg, E., LaCross, S., Strassman, A.M., 1994. The effects of clinically tested NMDA receptor antagonist memantine on carrageenin-induced thermal hyperalgesia in rats. Eur. J. Pharmacol. 255, 123–129.

Farkas, S., Ono, H., 1995. Participation of NMDA and non-NMDA excitatory amino acid receptors in the mediation of spinal reflex potentials in rats: An in vivo study. Br. J. Pharmacol. 114, 1193–1205.

Farkas, S., Tarnawa, I., Berzsenyi, P., Pátfalusi, M., Andrási, F., Ono, H., 1992. The role of non-*N*-methyl-D-aspartic acid exciatory amino acid receptors in mediation of spinal reflexes. In: Takai, K. (Ed.), Frontiers and New Horizons in Amino Acid Research. Elsevier, Amsterdam, pp. 471–475.

Ferreira, S.H., Lorenzetti, B.B., 1994. Glutamate spinal retrograde sensitization of primary sensory neurons associated with nociception. Neuropharmacology 33, 1479–1485.

Fundytus, M.E., Coderre, T.J., 1994. Effect of activity at metabotropic, as well as ionotropic (NMDA) glutamate receptors on morphine dependence. Br. J. Pharmacol. 113, 1215–1220.

Haley, J.E., Sullivan, A.F., Dickenson, A.H., 1990. Evidence for spinal *N*-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Res. 518, 218–226.

Harris, J.A., Corsi, M., Quartaroli, M., Arban, R., Bentivoglio, M., 1996. Upregulation of spinal glutamate receptors in chronic pain. Neuroscience 74, 7–12.

Hendershot, L.C., Forsaith, J., 1959. Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. J. Pharmacol. Exp. Ther. 125, 237–240.

Horváth, E.J., Hudák, J., Palkovits, M., Lenkei, Z., Fekete, M.I.K., Arányi, P., 1993. A novel specific binding site for homophtalazines in the rat brain. Eur. J. Pharmacol. 236, 151–153.

Hunter, J.C., Singh, L., 1994. Role of excitatory acid receptors in the mediation of the nociceptive response to formalin in the rat. Neurosci. Lett. 174, 217–221.

Hylden, J.L.K., Nahin, R.L., Traub, R.J., Dubner, R., 1989. Expansion of the receptive fields of spinal lamina I projection neurons in rats with unilateral adjuvant-induced inflammation: The contribution of dorsal horn mechanisms. Pain 37, 229–243.

Jackson, D.L., Graff, C.B., Richardson, J.D., Hargreaves, K.M., 1995. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. Eur. J. Pharmacol. 284, 321–325.

Kallman, M.J., Tizzaro, J.P., Modin, D.L., Griffey, K., Helton, D.R., 1995. Behavioral characterization of a noncompetitive AMPA antagonist LY-300164. XXV Ann. Meeting Soc. Neurosci. San Diego, CA, November 11–16, Abstr., p. 34.

Litchfield Jr., J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Malmberg, A.B., Yaksh, T.N., 1995. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 257, 1276–1279.

Mao, J., Price, D.D., Hayes, R.L., Lu, J., Mayer, D.J., 1992. Differential roles of *N*-methyl-D-aspartic acid and non-*N*-methyl-D-aspartic acid receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy. Brain Res. 598, 271–278.

Näsström, J., Karlsson, U., Post, C., 1992. Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. Eur. J. Pharmacol. 212, 21–29.

Newbould, B.B., 1963. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br. J. Pharmacol. 21, 127–136.

Palmer, A.J., Lodge, D., 1993. Cyclothiazide reverses the AMPA receptor antagonism of the benzodiazepine, GYKI 53655. Eur. J. Pharmacol. 244, 193–194.

Randall, L.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. 111, 409–419.

Ren, K., Hylden, J.L.K., Williams, G.M., Ruda, M.A., Dubner, R., 1992. The effects of non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. Pain 50, 331–344.

Schouenborg, J., Sjölund, B.H., 1986. First-order nociceptive synapses in rat dorsal horn are blocked by amino acid antagonists. Brain Res. 379, 394–398.

Sluka, K.A., Westlund, K.N., 1993. Centrally administered non-NMDA receptor antagonists block peripheral knee joint inflammation. Pain 55, 217–225.

Sluka, K.A., Willis, W.D., Westlund, K.N., 1993. Joint inflammation and hyperalgesia are reduced by spinal bicuculline. NeuroReport 5, 109–112.

Smith, E., Meldrum, B.S., 1990. Receptor site specificity for the acute effects of β-*N*-methylamino-alanine in mice. Eur. J. Pharmacol. 187, 131–134.

Song, X.-J., Zhao, Z.-Q., 1993. Differential effects NMDA and non-NMDA receptor antagonists on spinal cutaneous versus muscular nociception in the cat. NeuroReport 4, 17–20.

Stein, C., Millan, M.J., Yassouridis, A., Herz, A., 1988. Antinociceptive effects of μ- and κ-agonists in inflammation are enhanced by a peripheral opioid-specific mechanism. Eur. J. Pharmacol. 155, 255–264.

Székely, J.I., Máté, I., Török, K., Berzsenyi, P., Andrási, F., Tarnawa, I., 1995. Apparent antinociceptive and antiphlogistic effects of GYKI 52466, a 2,3-benzodiazepine, in some animal models. XXVI Int. Narcotics Res. Conf. Fife, Scotland, July 9–13, Abstr., p. 65.

Székely, J.I., Kedves, R., Török, K., Tarnawa, I., 1996. Further data on the apparent antinociceptive and antiphlogistic effects of 2,3-benzodiazepines. XXVII Int. Narcotics Res. Conf. Long Beach, California, July 21–26, Abstr., p. 96.

Tarnawa, I., Farkas, S., Berzsenyi, P., Pataki, Á., Andrási, F., 1989. Electrophysiological studies with a 2,3-benzodiazepine muscle relaxant: GYKI 52466. Eur. J. Pharmacol. 167, 193–199.

Tarnawa, I., Berzsenyi, P., Andrási, F., Botka, P., Hámori, T., Ling, I., Kôrösi, J., 1993. Structure–activity relationships of 2,3-benzodiazepine compounds with glutamate antagonistic action. Bioorg. Med. Chem. Lett. 3, 99–104.

Urban, L., Thompson, S.W.N., Dray, A., 1994. Modulation of spinal excitability: Cooperation between neurokinin and excitatory acid neurotransmitters. Trends Neurosci. 17, 432–438.

Witkin, L.B., Heubner, C.F., Gáldi, F., O'Keefe, E., Spitaletta, P., Plummer, A.J., 1961. Pharmacology of 2-amino-indane hydrochloride (SU-8629): A potent nonnarcotic analgesic. J. Pharmacol. Exp. Ther. 133, 400–408.

Woolf, C.J., Thompson, S.W.N., 1991. The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation: Implications for the treatment of post-injury pain hypersensitivity states. Pain 44, 293–299.

Xu, X.J., Hao, J.X., Seiger, A., Wiesenfeld-Halin, Z., 1993. Systemic excitatory acid receptor antagonists of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and of *N*-methyl-D-aspartate (NMDA) receptor relieve mechanical hypersensitivity after transient spinal cord ischemia in rats. J. Pharmacol. Exp. Ther. 267, 140–144.