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Ketotifen and Its Analogues Reduce Aversive Responding in the Rodent

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BARNES, N. M., B. COSTALL, M. E. KELLY, E. S. ONAIVI AND R. J. NAYLOR. *Ketotifen and its analogues reduce aversive responding in the rodent.* PHARMACOL BIOCHEM BEHAV **37**(4) 785–793, 1990.—The abilities of ketotifen and other 4-piperidylidene derivatives (HF200-184, HE36-953, SDZ209-321 and SDZ206-703) to inhibit aversive responding were compared in the mouse light/dark test box and in the rat social interaction test. Ketotifen and HF200-184 reduced aversive responding of the mouse to the brightly illuminated area of the test box and facilitated rat social interaction; HF200-184 was approximately 100 times more potent than ketotifen. The chronic administration and withdrawal from treatment with diazepam, ethanol, nicotine and cocaine in the mouse was associated with increased behavioural suppression which was prevented by the administration of ketotifen and HF200-184 during the period of withdrawal. HE36-953 also prevented the behavioural consequences of withdrawal from diazepam and cocaine. The relative potencies of ketotifen and its analogues to inhibit aversive responding did not correlate with their affinities for the 5-HT_3 recognition site. It is concluded that compounds within the 4-piperidylidene series can reduce behavioural suppression in rodent models of anxiety and attenuate the behavioural consequences of withdrawing from treatment with drugs of abuse.

Ketotifen analogues Aversive behaviour Drug withdrawal Mouse Rat

THE initial pharmacological studies on ketotifen revealed a potent antihistamine activity with antiallergic properties that have been used in the treatment of asthma (31). In later studies ketotifen was also shown to prevent changes in receptor sensitivity following perturbation in the β-adrenoceptor and dopamine receptor systems (2, 7–9). Such findings prompted a further investigation of the effects of ketotifen to prevent the behavioural consequences of withdrawal from drugs of abuse, since the latter are also associated with changes in catecholamine function (1, 19, 25, 33, 37, 39): ketotifen was shown to modify changes in aversive behaviour induced by withdrawal from treatment with diazepam, ethanol, nicotine and cocaine (17). In addition, ketotifen in its own right was shown to inhibit aversive responding in rodent models of anxiety (17).

The mechanism of action of ketotifen to cause such changes is not known, but 5-hydroxytryptamine is considered to play an important role in anxiety responding [see reviews (11) and (22)], a preliminary finding indicates that ketotifen has affinity for the 5-HT_3 receptor, albeit modest (17), and the 5-HT_3 receptor antagonist ondansetron is effective to inhibit in rodents the behavioural consequences of withdrawing from treatment with drugs of abuse (13). Here we investigate whether the behavioural actions of ketotifen are found in closely related compounds, and assess the significance of a 5-HT_3 receptor blockade.

METHOD

Experiments in the Mouse

Male albino BKW mice (Bradford strain), 30–35 g, were used throughout the studies. Mice were housed in groups of 10 in conditions of constant temperature (21°C) and controlled lighting (dark period 07.00–19.00 h) and fed ad lib on a standard laboratory chow. Water was available in the living cages at all times, and water containing ethanol was available to mice receiving ethanol treatment (see below).

Influence on Behaviour in the Light/Dark Test Box

Tests were conducted between 13.00 and 18.00 h in a quiet darkened room illuminated with a red light. Mice were taken from a dark holding room in a dark container to the dark testing room and allowed a 1-hour period of adaptation to the new environment. The test box (45 × 27 × 27 cm high) was open-topped and the base was lined into 9 cm squares, two-fifths painted black and illuminated by red light (1 × 60 W, 0 lux) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 1 × 60-W (400 lux) light source, the red and white lights being located 17 cm above the box. The compartments were connected by an opening 7.5 × 7.5 cm located at floor level in the centre of the partition. Mice were placed into the centre of the white, brightly lit area and the operator withdrew from the room. The mice were observed by remote video recording and four behaviours were noted, (a) the number of exploratory rearings in the white and black sections, (b) the number of line crossings in the white and black sections, (c) the time spent in the white and black areas and (d) the latency of the initial movement from the white to the black area.

Experimental Design

In the initial experiments mice received acute and chronic ad-

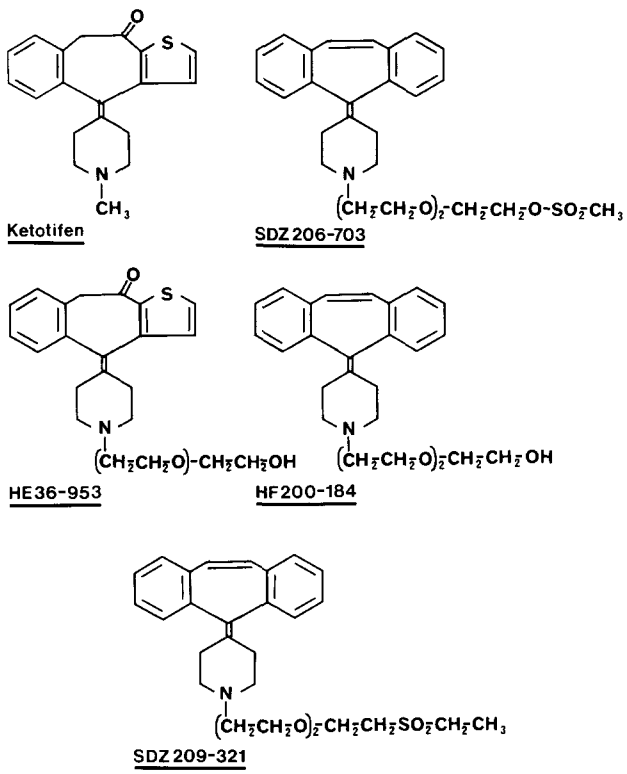

FIG. 1. The structures of ketotifen, HF200-184, HE36-953, SDZ206-703 and SDZ209-321.

ministrations of diazepam (IP b.i.d.), nicotine (IP b.i.d.), cocaine (IP b.i.d.) or ethanol (administered in the drinking water) to determine dose regimens suitable for chronic administration and withdrawal studies [detailed dose-response relationships have previously been established, see (4, 15, 16); Costall et al. unpublished data]. Ketotifen and its analogues HF200-184, HE36-953 and SDZ206-703 were administered intraperitoneally as acute 45-min pretreatments to establish dose regimes necessary to determine their effects during chronic administration and following withdrawal.

Subsequently, in the chronic drug administration (b.i.d. dosing at 08.00 and 20.00 h) and withdrawal experiments mice received (a) no treatment, (b) vehicle, (c) diazepam (10 mg/kg IP b.i.d. for 7 days), (d) ethanol (8% w/v in the drinking water for 14 days; ethanol intake per mouse approximated to 230 mg/24 h), (e) nicotine (0.1 mg/kg IP b.i.d. for 7 days) or (f) cocaine (1.0 mg/kg IP b.i.d. for 14 days) for behavioural assessment during treatment and following withdrawal. On the day of the last administration of diazepam, ethanol, nicotine and cocaine at 08.00 h, mice were treated with vehicle or ketotifen (10 mg/kg IP b.i.d.) or its analogues (1 mg/kg IP b.i.d.) at 08.00 and 20.00 h and on the following day(s) as appropriate to cover the period of drug withdrawal assessed after 8 or 48 h.

It is emphasised that mice were used once only. The results were analysed using single factor analysis of variance and, where appropriate, followed by Dunnett's procedure for comparing all treatments with control.

Experiments in the Rat

Male Sprague-Dawley rats, 225–275 g, were normally housed in groups of 5 under conditions of constant temperature (21°C) and kept on a 12-h light-dark cycle with lights on at 08.00 h. Animals were fed ad lib on a standard laboratory chow with water available at all times.

Influence on Rat Social Interaction

Tests were conducted between 13.00–18.00 h in an illuminated room. The apparatus used for the detection of changes in rat social interaction and exploratory behaviour consisted of an opaque white Perspex open-topped box (45 × 32 cm and 20 cm high) with 15 × 16 cm areas marked on the floor. Two naive rats, from separate housing cages, were placed into the unfamiliar box (with a 100-W bright white illumination 17 cm above) and their behaviour observed over a 10-min period by remote video recording. Two behaviours were noted, 1) social interaction between the animals was determined by timing (seconds), sniffing of partner, crawling under or climbing over partner, genital investigation of partner, following partner and 2) exploratory locomotion was measured as the number of crossings of the lines marked on the floor of the test box. Values for time spent in social interaction and moving around the observation cage were determined for individual animals. Animals were used in drug-treated pairs in treatment groups of six, i.e., twelve animals. Drug and vehicle treatments were administered intraperitoneally as a 40-min pretreatment. Data obtained were analysed using single factor analysis of variance followed by Dunnett's *t*-test.

Biochemistry

A 5-HT_3 receptor binding assay was performed on tissues taken from the entorhinal cortex of male Hooded-Lister rats using the methodology of Barnes et al. (3). Briefly, the tissues were dissected out on ice and pooled and homogenised (Polytron, setting 7 for 10 s) in 20 volumes of 50 mM Hepes buffer containing all the constituents of Krebs with a final pH of 7.4. The homogenate was centrifuged at 48,000 × g for 10 min at 4°C and the pellet was then resuspended and again centrifuged. The pellet was finally resuspended in the Hepes/Krebs buffer at a concentration of 0.2–0.3 mg protein ml^{-1}. Protein estimation was performed using the Bio-Rad Coomassie blue method using bovine serum albumin as the standard (Bradford, 1976). Assays were always performed on fresh tissue and carried out in replicates of three.

Six hundred and fifty μl of displacing drug or buffer (Hepes/Krebs) was added to assay tubes followed by 100 μl [^{3}H]zacopride (83 Ci/mmol) in Hepes/Krebs (final concentration 0.5 nM for competition studies or a range of concentrations from 0.05–5 nM for saturation studies); 250 μl of the brain tissue homogenate was added to initiate binding. The assay tubes were incubated for 30 min at 37°C and the incubation was terminated by rapid filtration through Whatman GF/B filters (presoaked in 0.1% vol./vol. polyethyleneimine) which were immediately washed with 9.6 ml of ice-cold Hepes/Krebs buffer (wash time 8 s). Each assay was completed within 60 min.

The filter discs were placed in 10 ml of 'Insta-gel' scintillant, left for dark adaptation for 6 h and radioactivity assayed by liquid scintillation counting. Results are the means ± s.e.m. of at least three separate experiments.

Drugs

Ketotifen fumarate [4,9-dihydro-4-(1-methyl-4-piperidylidene)-1OH-benzo[4,5]cyclohepta[1,2-b]thiophen-10-one], HF200-184 2 - [2 - [2[4 - (5H - dibenzo[a,d]cyclohept - 5 - ylidene) - 1 -piperidinyl]ethoxy]ethoxy]ethanol hydrogen fumarate, HE36-953 [4,9 -dihydro - 4 - [1 - [2 - [2 - (2 - hydroxyethoxy)ethoxy] - ethyl - 4 -

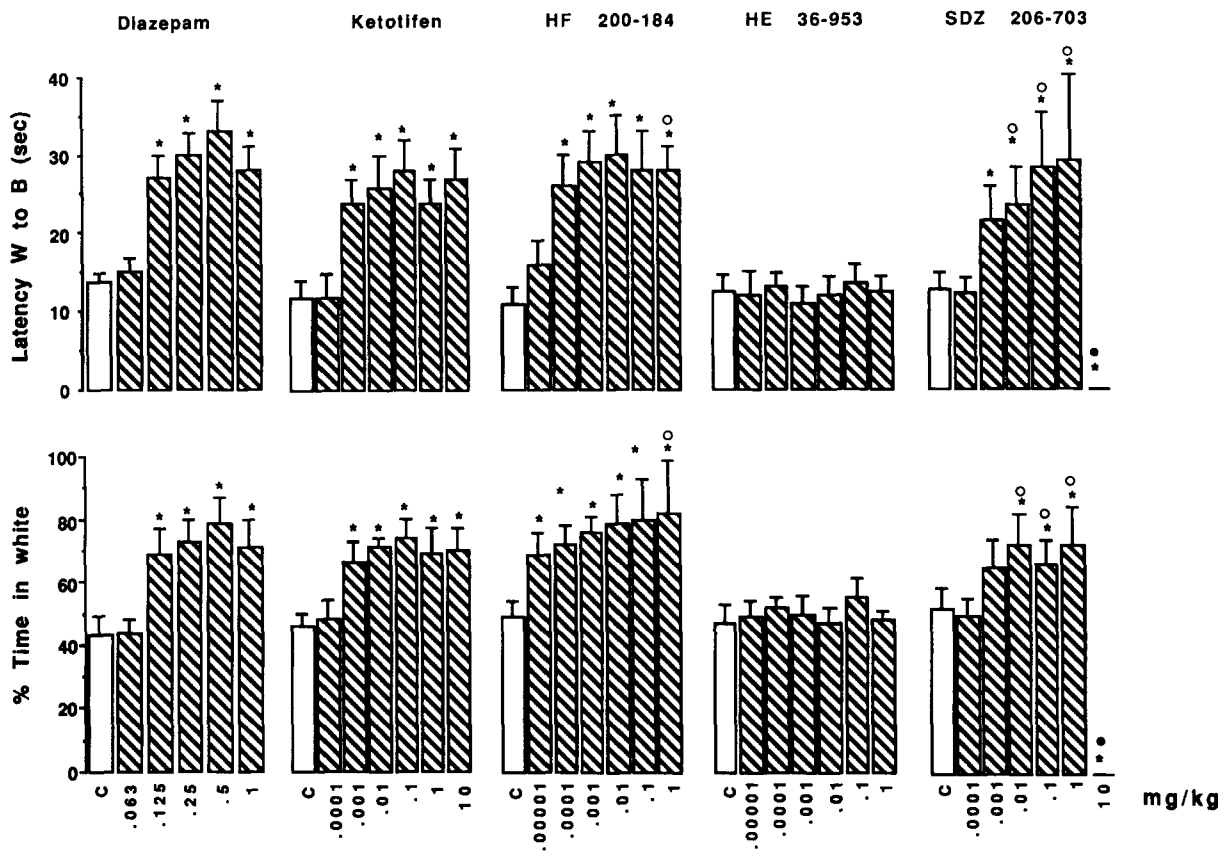

FIG. 2. The effects of diazepam, ketotifen and its analogues on mouse behaviour in the black and white test box. Behavioural changes in the latency of movement from the white (W) to the black (B) section (after first placement into the white area) and % time spent in the white area during the 5-min observation period were recorded. Data obtained from control (C) and drug-treated mice were analysed using single factor analysis of variance and Dunnett's *t*-test. Significant increases or decreases in responding are indicated as $*p<0.01$. n = 10. Vertical bars indicate S.E.M.s and were calculated on the original data for calculation of the % time in the white section. ○Sedation, ●Toxic.

piperidinylidene]-1OH-benzo[4,5]cyclohepta [1,2-6]thiophene-10-one] hydrogen bromide, SDZ209-321, (4-(5H-dibenzo[a,d] cyclohept-5-ylidene)-1-[2-[2-ethylsulfonyl-ethoxy)ethoxy]-ethyl]-piperidin hydrogen fumarate, SDZ206-703 [4-(5H-dibenzo[a,d] cyclohept-5-yliden)-1-[2-[2-(2mesyloxy-ethoxy)-ethyl]-piperiden] hydrogen fumarate (Sandoz, Basel, Switzerland), nicotine hydrogen tartrate (BDH Ltd.), cocaine hydrochloride (BDH Ltd.) were prepared daily in distilled water. Drinking water containing ethanol (J. Burroughs Ltd.) was freshly prepared each day. Diazepam (Roche) was prepared in the minimum quantity of polyethylene glycol made up to volume with distilled water. Doses are expressed as the base and, with the exception of ethanol, were administered in a volume of 1 ml/100 g body weight (mouse) or 1 ml/kg body weight (rat).

RESULTS

A Comparison of the Effects of Acute Administration of Diazepam, Ketotifen and Its Analogues in the Mouse

Control mice placed into the white section of the test box moved within 12 to 14 s into the black compartment, subsequently moving between the dark and light areas and spending approximately 50% of their time in each compartment. The administration of diazepam delayed entry into the dark compartment and increased the proportion of time mice spent in the light compartment (Fig. 2). Rearings and line crossings in the white area increased concomitantly and decreased in the black area [see also Costall et al. (14)]. Therefore, these values are not reported unless changes occurred that reflected nonspecific alterations in behaviour.

Ketotifen and HF200-184 caused an identical change to preferentially increase behaviour in the light section, but were approximately 100 and 10,000 times respectively more potent than diazepam. Such changes in behaviour were not associated with nonspecific changes in behaviour, with the exception of the highest dose of 1 mg/kg HF200-184 which caused sedation (line crossings were reduced in both the black and white sections by 94 and 86% respectively, $p<0.001$). Similar nonspecific reductions in activity were observed using SDZ206-703 (0.01–10.0 mg/kg) (line crossings were reduced in both sections by 61–92% $p<0.01$–0.001), to interfere with normal movement from the light to the black compartment and the time spent in the two compartments; the highest dose of 10 mg/kg was toxic to all animals. The administration of HE36-953 (0.00001–1.0 mg/kg) failed to modify mouse behaviour in the test box (Fig. 2); SDZ209-321 (0.00001–1.0 mg/kg) also failed to modify behaviour (data not shown).

A Comparison of the Effects of Acute Administration of Diazepam, Ketotifen and Its Analogues in Rat Social Interaction

Vehicle-treated rats spent approximately 70 s in social interaction in a 10-min period under high light unfamilar conditions.

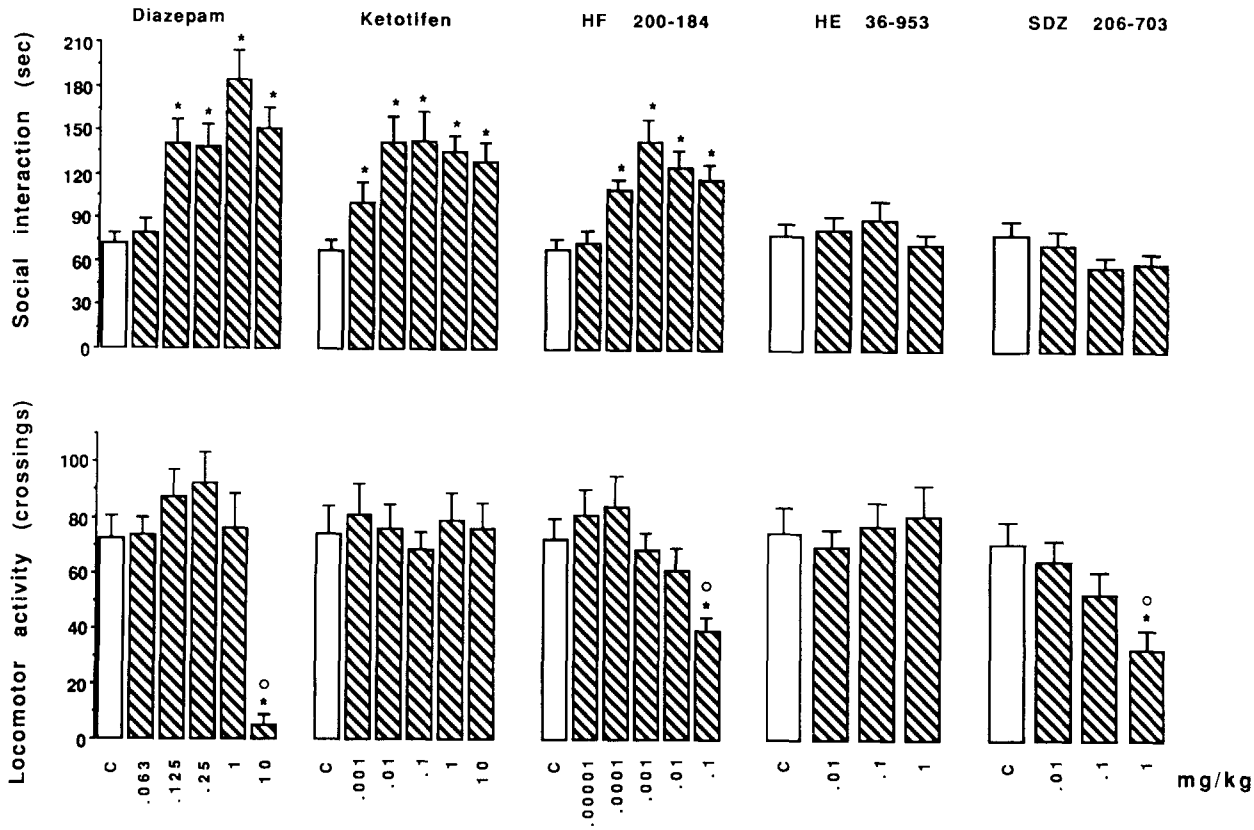

FIG. 3. The effects of diazepam, ketotifen and its analogues in the social interaction test in the rat. The time spent in active social interaction between pairs of rats was recorded during a 10-min period under the experimental conditions of high light and unfamiliarity of rats with each other and the test box. Crossings of lines marked on the test box floor were also noted. Data obtained in control (C) and drug-treated rats was analysed by single factor analysis of variance followed by Dunnett's *t*-test. n=6. S.E.M.s given. Significant increases/decreases in responding are indicated (*$p<0.05$–$p<0.001$). °Sedation.

The administration of diazepam (0.125–1.0 mg/kg), ketotifen (0.001–10 mg/kg) and HF200-184 (0.0001–0.01 mg/kg) increased social interaction with no change in activity. However, activity was reduced using a higher dose of diazepam (10 mg/kg), HF200-184 (0.1 mg/kg) and SDZ206-703 (1.0 mg/kg). HE36-953 and SDZ206-703 failed to modify social interaction (Fig. 3); SDZ209-321 (0.01–1.0 mg/kg) also failed to modify social interaction in the rat (data not shown).

The Effects of Chronic Treatment With Diazepam, Ethanol, Nicotine and Cocaine: Interaction With Ketotifen and Its Analogues in the Mouse

Drug regimens of diazepam (10 mg/kg IP b.i.d. for 7 days), ethanol [8% w/v in the drinking water 14 days; equivalent to a daily intake per mouse of approximately 7.5 g/kg ethanol, see also (4)], nicotine (0.1 mg/kg b.i.d. for 7 days) and cocaine (1.0 mg/kg b.i.d. for 14 days) were selected on the basis of previous work (4, 15, 16) showing that their administration to mice increased the proportion of time spent in the white compartment and the latency of the first movement from the white to the black section (see Figs. 4 to 7). Thus, the profile of behavioural change caused by the chronic drug administrations was the same as that caused by an acute administration of diazepam. However, within hours of withdrawal from treatment with diazepam, ethanol, nicotine or cocaine the preference of mice to explore the white compartment was reversed to an exploration of the dark area. The changes in behaviour caused by withdrawal from the 4 drugs were similar, mice moving more rapidly into the black compartment and spending a greater proportion of time in the dark area (Figs. 4 to 7). The changes were maximally developed for at least 2 days (diazepam and ethanol) or 4 days (nicotine and cocaine); the ability of ketotifen and its analogues to modify the behavioural consequences of drug withdrawal were assessed at times of a stable and marked behavioural change; 8 h for diazepam and cocaine, and 48 h for alcohol and nicotine. The administration of ketotifen (10 mg/kg IP b.i.d.) and HF200-184 (1 mg/kg IP b.i.d.) during the period of withdrawal from ethanol or nicotine prevented the exacerbation in aversive responding, and indeed, using HF200-184, the profile of change was reversed to an increase in time spent in the light area. The administration of HE36-953 and SDZ206-703 (1 mg/kg IP b.i.d.) failed to modify the behavioural consequences of withdrawal from either ethanol or nicotine (Figs. 5 and 6).

The administration of ketotifen (10 mg/kg IP) and HF200-184 (1 mg/kg IP) also prevented the behavioural consequences of withdrawal from cocaine and diazepam; HF200-184 again reversed the profile of change to an increase in time spent in the light area. The administration of HE36-953 (1 mg/kg IP) also prevented the behavioural consequences of withdrawal from diazepam and cocaine (cf. ethanol and nicotine withdrawal): SDZ206-703 was ineffective (Figs. 4 and 7). SDZ209-321 (1.0 mg/kg) failed to prevent the behavioural consequences of withdrawal from treatment with diazepam, ethanol, nicotine and cocaine (data not shown).

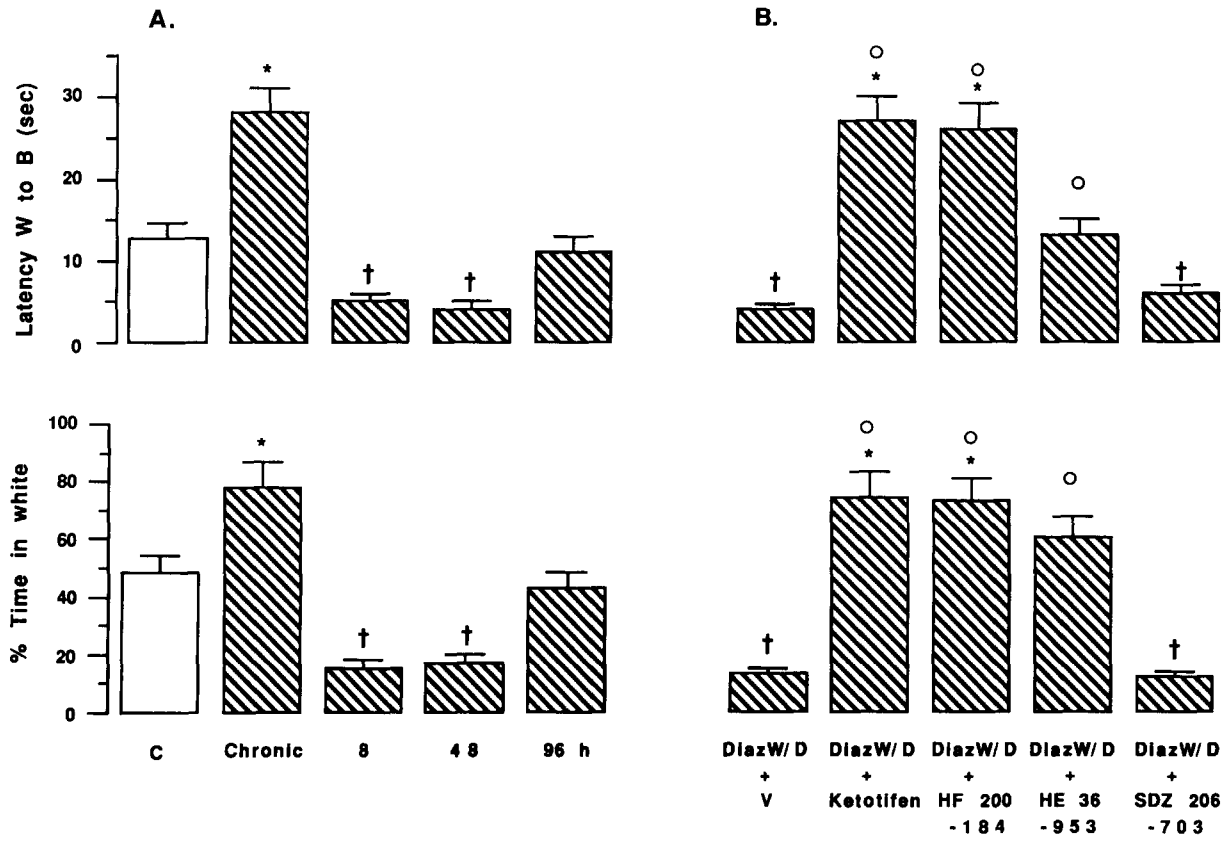

FIG. 4. The effects on mouse exploration in the light/dark test box of (A) chronic treatment with diazepam (10 mg/kg IP b.i.d. 7 days) and effects of withdrawal from treatment (Diaz. W/D) at 8, 48 and 96 h and (B) the influence of ketotifen (10 mg/kg IP) (Diaz. W/D + ketotifen) and analogues of ketotifen (1 mg/kg IP) (Diaz. W/D + HF200-184/HE36-953/SDZ206-703) on the behavioural consequences of withdrawal from diazepam at 8 h, assessed as the latency of the first movement from the white (W) to the black (B) compartment and the % time spent in the white compartment during the 5-min observation period. *$p<0.05$–0.001 for redistribution of behaviour in favour of the light section and † for distribution in favour of the dark section as compared to vehicle-treated controls (C). °$p<0.001$ for inhibition of the behavioural consequences of withdrawing from treatment with diazepam (single factor analysis of variance followed by Dunnett's *t*-test). n = 6. S.E.M.s are given and calculated from original data.

The Effect of Ketotifen and Its Analogues in a 5-HT$_3$ Receptor Radioligand Binding Assay

[^{3}H]Zacopride is a highly selective 5-HT$_3$ receptor antagonist (36) and has been used to identify 5-HT$_3$ recognition sites in the rodent brain (3). At the low nanomolar concentrations employed, it would not be expected to influence the 5-HT$_4$ receptors. In the present experiments using homogenates of rat entorhinal cortex [^{3}H]zacopride (0.05–5.0 nM) displayed saturable binding as measured by the inclusion of the 5-HT$_3$ receptor antagonist granisetron (10 μM) in the incubation media. A Scatchard transformation gave values of K_D 0.74 ± 0.05 nM and B_{max} 91 ± 6 fmol/mg protein (mean ± S.E.M., n = 11) with Hill Slopes close to unity. Granisetron in low nanomolar concentrations-displaced approximately 40–50% of the total [^{3}H]zacopride (0.5 nM) binding: ketotifen also caused a concentration-related displacement of [^{3}H]zacopride binding, although it was some 1000 times less potent than granisetron. HF200-184 and HE36-953 also displaced [^{3}H]zacopride and were approximately 5 to 10 times less potent than ketotifen (Fig. 8).

Granisetron in low nanomolar concentration competed for approximately 40–50% of the total [^{3}H]zacopride (0.5 nM) binding (pki = 8.81 ± 0.09, mean ± S.E.M., n = 5, Fig. 8): ketotifen, HF200-184 and HE36-953 also competed with [^{3}H]zacopride binding to a similar level as granisetron, although with weaker affinity (pki = 6.51 ± 0.10, 5.82 ± 0.13 and 5.27 ± 0.04, respectively, mean ± S.E.M., n = 3, Fig. 8).

DISCUSSION

Anxiolytic agents from the benzodiazepine series inhibit suppressed behaviour in the light/dark test in the mouse and social interaction test in the rat (14, 18, 20). The present study has confirmed that ketotifen has a similar profile of action in both the mouse and rat tests (17) and extended the observation to HF200-184 which was found to be 100 times more potent than ketotifen. The substitution on the nitrogen would appear to be important, since activity was observed in the presence of the ethoxy-ethoxy-ethanol grouping (HF200-184), but was lost with the ethoxy-ethoxy-ethyl-sulfonyl (SDZ209-321) and ethoxy-ethoxy-ethoxy-methanesulfonyl (SDZ206-703) substitutions. However, the presence of the ethoxy-ethoxy-ethanol grouping in HE36-953 failed to confer activity, indicating that changes in the benzocycloheptathiophene structure to include a phenyl substitution are also important. It remains possible that the sedative potential of SDZ206-703 may have obscured an ability to inhibit suppressed behaviour.

Withdrawal from chronic treatment with diazepam, ethanol, nicotine and cocaine cause changes in behaviour opposite to the acute administration of anxiolytic agents and ketotifen, i.e., an exacerbation in aversive responding in the light/dark test in the

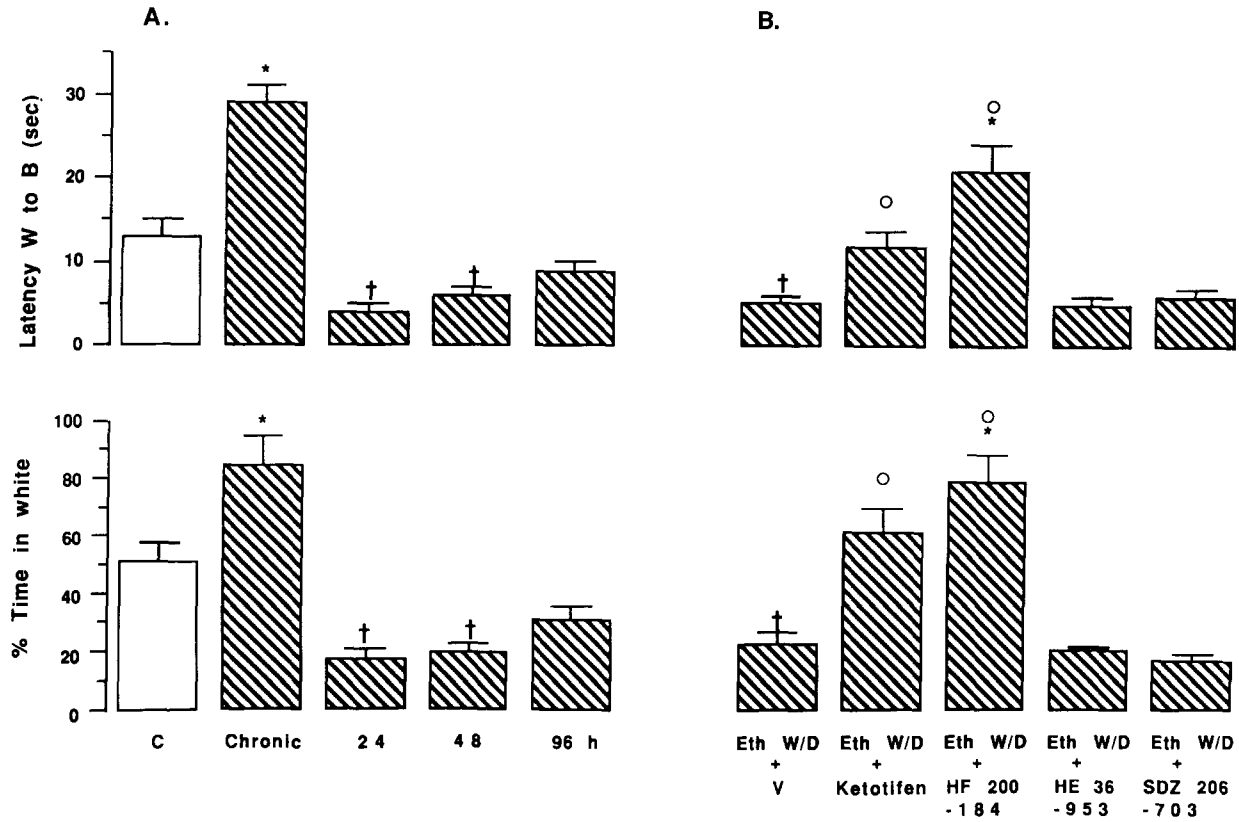

FIG. 5. The effects on mouse exploration in the light/dark test box of (A) chronic treatment with ethanol (8% w/v in the drinking water for 14 days) and effects of withdrawal from treatment (Eth. W/D) at 24, 48 and 96 h and (B) the influence of ketotifen (10 mg/kg IP) (Eth. W/D + ketotifen) and analogues of ketotifen (1 mg/kg IP) (Eth. W/D + HF200-184/HE36-953/SDZ206-703) on the behavioural consequences of withdrawal from ethanol at 48 h, assessed as the latency of the first movement from the white (W) to the black (B) compartment and the % time spent in the white compartment during the 5-min observation period. *$p<0.05$–0.001 for redistribution of behaviour in favour of the light section and † for distribution in favour of the dark section as compared to vehicle-treated controls (C). °$p<0.001$ for inhibition of the behavioural consequences of withdrawing from treatment with ethanol (single factor analysis of variance followed by Dunnett's t-test). n=6. S.E.M.s are given and calculated from original data.

mouse (4, 15, 16). The present study confirms that the administration of ketotifen during the period of withdrawal from treatment with drugs of abuse can prevent the increase in aversive responding (17). The study indicates that HF200-184 can also prevent the behavioural consequences of drug withdrawal, and it is possible that both ketotifen and HF200-184 are superimposing their disinhibitory effects upon a suppressed behaviour. However, whilst the disinhibitory effects of ketotifen and HF200-184 may play a contributory role, it is of interest that HE36-953 failed to modify aversive behaviour in its own right yet retained an ability to antagonise the behavioural consequences of withdrawal from treatment with diazepam and cocaine. This would indicate that an unspecified action of HE36-953 and perhaps other analogues may moderate aversive responding following withdrawal from diazepam and cocaine.

The results obtained with HE36-953 were of further interest since it failed to modify the behavioural consequences following withdrawal from treatment with ethanol and nicotine. Whilst the failure may reflect the use of too low a dose of HE36-953 (the use of higher doses was made difficult by the development of sedation), it may question whether the effects of withdrawal from ethanol and nicotine are mediated via pathways different to those influenced by diazepam and cocaine. Yet the actions of diazepam are mediated via the benzodiazepine-GABA receptor-chloride channel complex and ethanol may also exert at least some of its effects via this site [see reviews (23, 30, 34, 38)]. In turn, this may modify noradrenaline, dopamine and 5-hydroxytryptamine function. Cocaine can exert a more direct effect to modify monoamine transmission and its actions on the 5-hydroxytryptamine and dopamine systems may influence the consequences of withdrawal from treatment [see review (29); also (19, 26, 33)]. But nicotine is also known to modify monoamine function (5, 6, 21, 25) and there is no immediate explanation for the actions of HE36-953 to differentially modify the consequences of withdrawal from treatment with diazepam and cocaine, or ethanol and nicotine. However, it should be noted that HE36-953 modified the consequences of cocaine and diazepam withdrawal when assessed after an eight-hour withdrawal period, whereas its effects on nicotine and ethanol withdrawal were studied after a forty-eight-hour period. It is possible that transmitter systems which mediate the withdrawal response are different at different time points after withdrawal, and this is worthy of further investigation.

However, there is considerable evidence for a role of 5-hydroxytryptamine in aversive behaviour [see reviews (11) and (22)] and the ability of ketotifen and its analogues to reduce the behavioural consequences of withdrawal from treatment with drugs of abuse mimics the action of the 5-HT_3 receptor antagonist ondansetron (13). The latter compound inhibits aversive responding

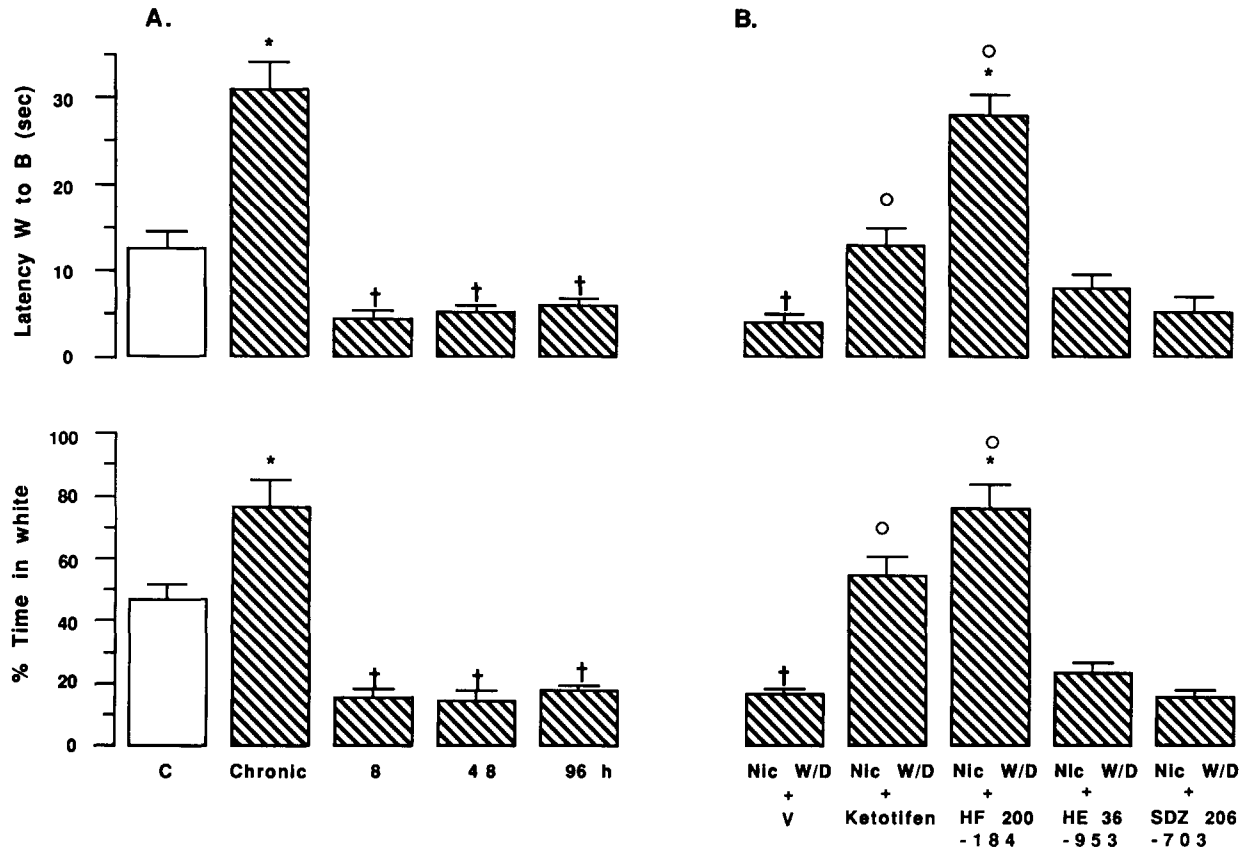

FIG. 6. The effects on mouse exploration in the light/dark test box of (A) chronic treatment with nicotine (0.1 mg/kg IP b.i.d. 7 days) and effects of withdrawal from treatment (Nic. W/D) at 8, 48 and 96 h and (B) the influence of ketotifen (10 mg/kg IP) (Nic. W/D + ketotifen) and analogues of ketotifen (1 mg/kg IP) (Nic. W/D + HF200-184/HE36-953/SDZ206-703) on the behavioural consequences of withdrawal from nicotine at 48 h, assessed as the latency of the first movement from the white (W) to the black (B) compartment and the % time spent in the white compartment during the 5-min observation period. *$p<0.05$–0.001 for redistribution of behaviour in favour of the light section and † for distribution in favour of the dark section as compared to vehicle-treated controls (C). °$p<0.001$ for inhibition of the behavioural consequences of withdrawing from treatment with nicotine (single factor analysis of variance followed by Dunnett's t-test). n = 6. S.E.M.s are given and calculated from original data.

(28) and may also affect the reward system (10, 12, 24, 27, 32, 35); such actions may contribute importantly to the actions of 5-HT_3 receptor antagonists to influence the behavioural consequences of withdrawal from treatment with drugs of abuse. Thus, the 5-HT system may provide an important common site of action for 5-HT_3 receptor antagonists and ketotifen and its analogues to modify behaviour, and the similarity in the behavioural profile of the two groups of compounds prompted an assessment of ketotifen and related agents as potential 5-HT_3 receptor antagonists. Ketotifen was found to have affinity for the 5-HT_3 receptor in radioligand binding assays using the 5-HT_3 receptor antagonist [^{3}H]zacopride. However, the effects of ketotifen were achieved at micromolar concentrations and HF200-184, although two orders of magnitude more potent than ketotifen to modify behaviour, was five to ten times less potent in the 5-HT_3 binding assay. A further discrepancy between the binding and behavioural data was obtained using HE36-953 which failed to modify aversive responding in its own right, yet was the same order of potency as HF200-184 in the binding assay. Therefore, whilst a 5-HT_3 receptor antagonist effect may contribute to the behavioural actions of ketotifen and its analogues, it is unlikely to play a key role.

Thus, derivatives from the 4-piperidylidene series are revealed as potent agents to inhibit aversive behaviour in rodent models and to prevent the behavioural consequences of withdrawing from treatment with drugs of abuse. Their mechanism(s) of action remains to be determined and may offer new insights into the systems moderating aversive behaviour and the behavioural changes following withdrawal from drugs of abuse.

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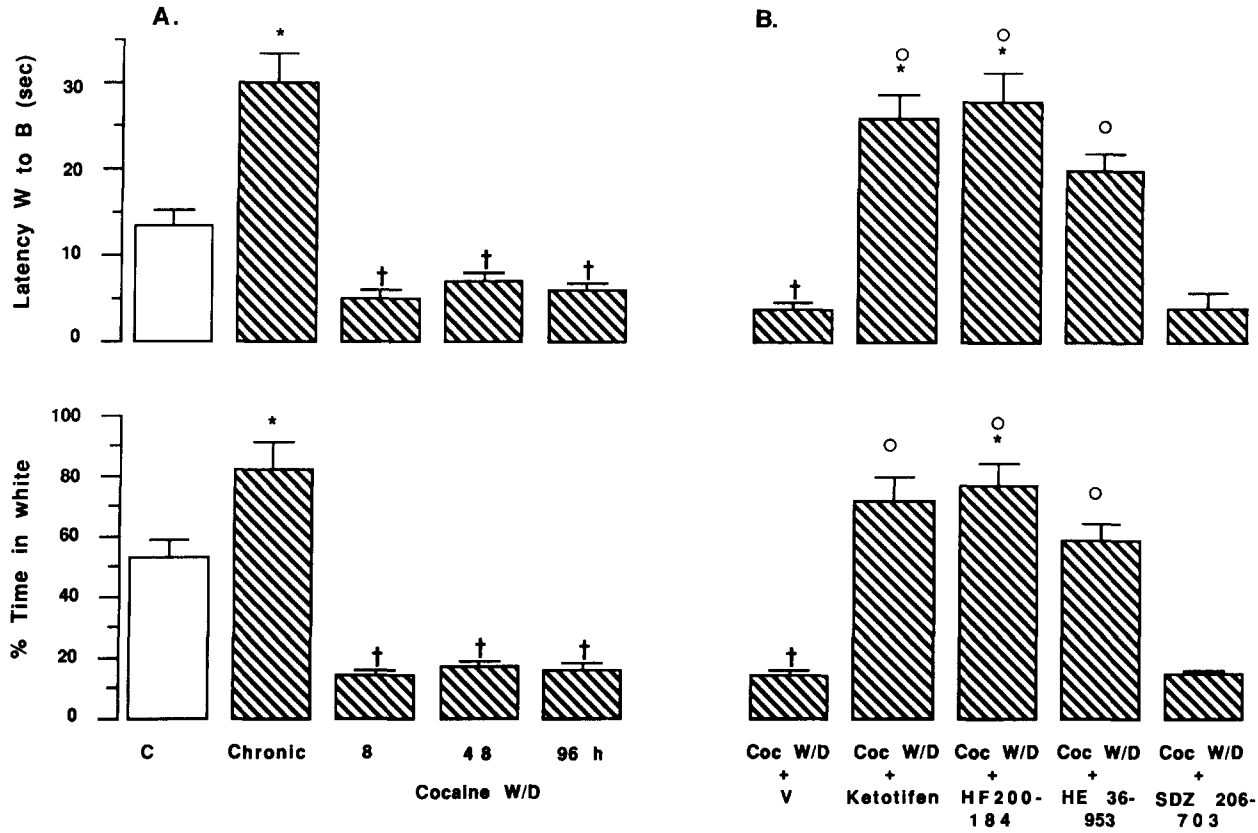

FIG. 7. The effects on mouse exploration in the light/dark test box of (A) chronic treatment with cocaine (1 mg/kg IP b.i.d. 14 days) and effects of withdrawal from treatment (Coc. W/D) at 8, 48 and 96 h and (B) the influence of ketotifen (10 mg/kg IP) (Coc. W/D + ketotifen) and analogues of ketotifen (1 mg/kg IP) (Coc. W/D + HF200-184/HE36-953/SDZ206-703) on the behavioural consequences of withdrawal from cocaine at 8 h, assessed as the latency of the first movement from the white (W) to the black (B) compartment and the % time spent in the white compartment during the 5-min observation period. *$p<0.05$–0.001 for redistribution of behaviour in favour of the light section and † for distribution in favour of the dark section as compared to vehicle-treated controls (C). °$p<0.001$ for inhibition of the behavioural consequences of withdrawing from treatment with cocaine (single factor analysis of variance followed by Dunnett's t-test). n = 6. S.E.M.s are given and calculated from original data.

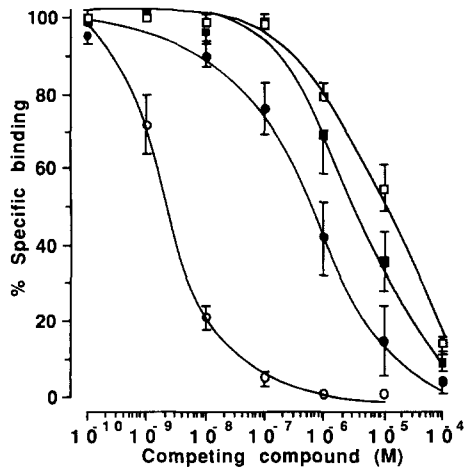

FIG. 8. The abilities of granisetron (○), ketotifen (●) and its analogues HF200-184 (■) and HE36-953 (□) to compete for [^{3}H]zacopride (0.5 nM) binding in homogenates of the rat entorhinal cortex. Results are the means ± S.E.M. of 3 separate experiments. The pKi values were 8.81 ± 0.09, 6.51 ± 0.10, 5.82 ± 0.13 and 5.27 ± 0.04 for granisetron, ketotifen, HF200-186 and HE36-953, respectively (mean ± S.E.M.).

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