Pharmacology Biochemistry and Behavior, Vol. 60, No. 3, pp. 703–707, 1998
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0091-3057/98 $19.00 + .00

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PII S0091-3057(98)00043-4

The Discriminative Stimulus Effects of KA 672, a Putative Cognitive Enhancer: Evidence for a 5-HT_{1A} Component

J. C. WINTER, S. E. HELSLEY AND RICHARD A. RABIN

Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, 102 Farber Hall, Buffalo, NY 14214

Received 25 July 1997; Revised 16 December 1997; Accepted 30 December 1997

WINTER, J. C., S. E. HELSLEY AND R. A. RABIN. *The discriminative stimulus effects of KA 672, a putative cognitive enhancer: Evidence for a 5-HT_{1A} component*. PHARMACOL BIOCHEM BEHAV **60**(3) 703–707, 1998.—Stimulus control was established in a group of seven rats using a dose of KA 672 [7-methoxy-6-{3-[4-(2-methoxyphenyl)-1-piperazinyl] propoxy}3,4-dimethyl-2H-1-benzopyran-2-one HCl] of 1.0 mg/kg, administered IP, 15 min before training. A two-lever operant task using a fixed-ratio 10 schedule of sweetened milk reinforcement was used. Based upon a criterion for the presence of stimulus control of five consecutive sessions during which 83% or more of all responses were on the appropriate lever, a mean of 23 sessions was required to reach criterion performance. Subsequently, it was observed that KA 672-induced stimulus control is partially but significantly antagonized by the selective 5-HT_{1A} antagonist, WAY-100635. Furthermore, KA 672 generalized to the selective 5-HT_{1A} agonist, 8-hydroxy-dipropylaminotetralin [8-OH-DPAT], and this generalization was blocked by WAY-100635. Other tests of generalization were conducted with the structural analogs, scoparone, CD-127, and OMPP, as well as with the receptor-selective ligands ketamine, PCP, dizocilpine, prazosin, urapidil, apomorphine, and DTG. Of these drugs only dizocilpine met the criteria for full substitution while an intermediate level of generalization was observed to ketamine, PCP, urapidil, and apomorphine. The present results indicate that KA 672-induced stimulus control is mediated in part by activity at the 5-HT_{1A} receptor and that behaviorally significant interactions occur as well at PCP/NMDA, dopaminergic, and adrenergic receptors. © 1998 Elsevier Science Inc.

KA 672 Aging Rat Drug-induced stimulus control 8-OH-DPAT WAY-100635

KA 672 (Fig. 1) is currently under development as a cognitive enhancer and as a treatment for dementia. In rats and mice, KA 672 has been reported to enhance acquisition and retention of both active and passive avoidance responses (12). In their recent review of KA 672, Noldner et al. (16) point out multiple pharmacological actions that might be relevant to antidementia activity. Prominent among these are inhibition of acetylcholinesterase, protection against NMDA-induced toxicity, and nanomolar affinity for dopaminergic (D_2), serotonergic (5-HT_{1A}), adrenergic (alpha$_1$), and sigma receptors.

Drug discrimination is a sensitive technique for studying the interoceptive states produced in animals by drugs acting upon the central nervous system [for reviews, see (3,7,19)]. After it is demonstrated that a given drug can establish stimulus control, one can proceed to characterize the drug in terms of other pharmacological agents that mimic, antagonize, or modulate its discriminative effects. Although exceptions have been noted (1,2), a variety of studies of psychoactive drugs in animals has revealed a remarkable correlation between drug-induced stimulus control and subjective effects in humans (4,8,17,20).

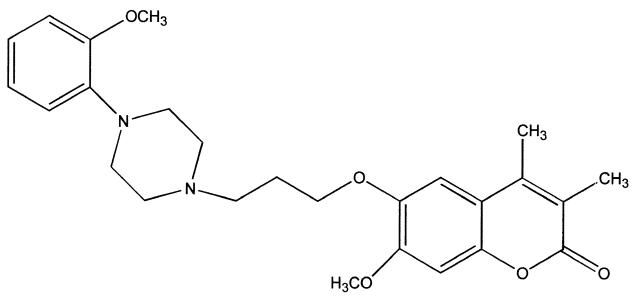
In the present investigation, it was first demonstrated that stimulus control could be established using KA 672. Pharmacological characterization of the drug was then attempted using, as an initial guide, previous investigations of the drug as cited above.

METHOD

Animals

Male Fischer-344 rats were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) at an age of

Requests for reprints should be addressed to Dr. J. C. Winter, State University of NY at Buffalo, School of Medicine and Biomedical Sciences, Department of Pharmacology & Toxicology, 102 Farber Hall, Buffalo, NY 14214-3000.



KA 672

FIG. 1. The structure of KA 672 [7-methoxy-6-{3-[4-(2-methoxyphenyl)-1-piperazinyl] propoxy}3,4-dimethyl-2H-1-benzopyran-2-one HCl].

approximately 6 weeks. They were housed in pairs under a natural light–dark cycle and allowed free access to water in the home cage. All handling and testing occurred during daytime hours. Standard rat chow was provided immediately following training sessions. Caloric intake was controlled so as to maintain adult body weights of approximately 300 g.

Apparatus

Three small animal test chambers (Coulbourn Instruments model E 10-10) were used for all experiments. These were housed in larger light-proof, sound-insulated boxes that contained a house light and an exhaust fan. Chambers contained two levers mounted at opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water. Sessions were managed by a microcomputer using operant control software (Coulbourn Instruments D91-12, version 4.0).

Procedure

Training. After learning to drink from the dipper, rats were trained to press first one and then the other of the two levers. The number of responses for each reinforcement was gradually increased from 1 to 10. During this time, the reinforced lever was alternated on a random basis. All subsequent training and testing sessions used a fixed-ratio 10 (FR10) schedule of reinforcement. Discrimination training was then begun. Fifteen minutes before each 10-min training session, subjects were injected IP with either saline or KA 672 (1.0 mg/kg). Following the administration of KA 672, every 10th response on the KA 672-appropriate lever was reinforced. Similarly, responses on the saline-appropriate lever were reinforced on a FR10 schedule following the injection of saline. For half of the subjects, the left lever was designated as the KA 672-appropriate lever. During discrimination training, KA 672 and saline were alternated on a daily basis. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever.

After stimulus control with KA 672 was well established, substitution tests and antagonism tests were conducted once per week in each animal so long as performance during the remainder of the week did not fall below a criterion level of 83% correct responding. Subjects were assigned to test groups with the intention of including equal numbers of those trained on the previous day with saline and drug, respectively. During test sessions, no responses were reinforced and the session was terminated after the emission of 10 responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing total number of responses emitted prior to lever selection, that is, prior to the emission of 10 responses on either lever, by elapsed time.

In substitution tests various doses of drugs were administered by IP injection 60 min prior to testing. In antagonism tests, various doses of drugs and 1.0 mg/kg of KA 672 were administered by IP injection 60 and 15 min prior to the initiation of test sessions, respectively. Response rates and drug-appropriate responding were recorded as described above.

Drugs

KA 672 [7-methoxy-6-{3-[4-(2-methoxyphenyl)-1-piperazinyl] propoxy}3,4-dimethyl-2H-1-benzopyran-2-one HCl], OMPP [1-(2-methoxyphenyl)piperazine HCl], and CD-127 [6-(3-hydroxypropoxy)-7-methoxy-3,4-dimethyl-2H-1-benzopyran-2-one] were obtained from Dr. S. S. Chatterjee, Department of Pharmacology, Dr. Willmar Schwabe GmbH & Co., Karlsruhe, Germany. 8-Hydroxy-dipropylaminotetralin HCl [8-OH-DPAT], ketamine, (+)-MK-801 hydrogen maleate [dizocilpine], prazosin HCl, urapidil HCl, R(−)-apomorphine HCl, phencyclidine HCl, 5-methoxy-*N,N*-diethyltryptamine oxalate [5-MeO-DMT], and DTG [1,3-di(2-tolyl)guanidine] were purchased from Research Biochemicals International, Natick, MA. WAY-100635 was generously provided by Wyeth-Ayerst Research, Princeton, NJ. With the exception of WAY-100635, which was injected SC, all drugs were dissolved in sterile deionized water and were administered IP in a volume of 1 ml/kg body weight.

Statistical Analysis

Because it has been our experience that stimulus control data expressed as percent drug-appropriate responding often fail a test of normality, hence precluding parametric statistical analysis, the present data were subjected to a square root transformation prior to analysis. In most instances, a normal distribution resulted and the data were then tested using one-way repeated measures analysis of variance with subsequent pairwise multiple comparisons by the method of Student–Newman–Keuls. If, as sometimes occurred, the transformed data failed either a test of normality or a test of equal variance, one-way repeated-measures ANOVA on ranks was used. Differences were considered to be statistically significant if the probability of their having arisen by chance was <0.05. All analyses were conducted using SigmaStat for Windows™ (Jandel Scientific Software, San Rafael, CA). Criteria for generalization and antagonism were as previously described (22).

RESULTS

A group of seven rats trained with KA 672 at a dose of 1.0 mg/kg (IP) with a 15-min pretreatment time required a mean of 23 sessions (range = 17–36) to reach criterion performance. Over the 12-month course of the present experiments, stimulus control was reliably maintained in five of the subjects. Figure 2 shows an orderly dose–response relationship for KA 672 with no significant effects upon the rate of responding. Illustrated in Fig. 3 is the time course for stimulus control by KA

672. Also shown in Fig. 2 are the effects upon KA 672-induced stimulus control of the selective 5-HT_{1A} antagonist, WAY-100635 (9). Both at the training dose of KA 672 (1.0 mg/kg) and at a dose of KA 672 of 0.3 mg/kg, an intermediate level of antagonism was produced, i.e., the degree of KA 672-induced stimulus control was significantly different from both training conditions.

To further explore the possible role of 5-HT_{1A} receptors in the stimulus effects of KA 672, generalization of KA 672 to the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, was tested. As is seen in Fig. 4, at a dose of 0.2 mg/kg, 8-OH-DPAT met the criteria for complete substitution. Furthermore, both KA 672-appropriate responding and rate suppression induced by this dose of 8-OH-DPAT were completely antagonized by WAY-100635.

In Table 1 are shown the results of preliminary studies intended (a) to explore the structural requirements for KA 672-induced stimulus control, and (b) to identify receptor interactions, other than those of the 5-HT_{1A} type, which might be involved in KA 672-induced stimulus control. With respect to structural requirements, the absence of generalization of KA 672 to the coumarin analogs, scoparone and CD-127, or to OMPP, suggests that both the phenylpiperazine and coumarin moieties are essential for KA 672-induced stimulus control. Of the remaining drugs in Table 1, only dizocilpine met the criteria for full substitution for KA 672 but did so only at a dose that completely suppressed responding in four of seven

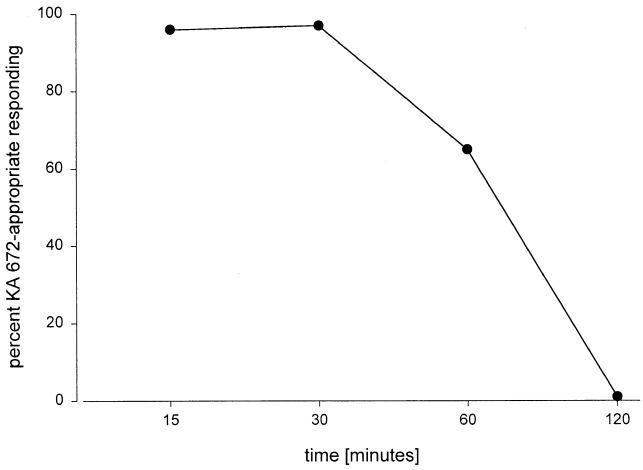


FIG. 3. Time course of stimulus control by KA 672 in rats (n = 5) trained with KA 672 as a discriminative stimulus (1.0 mg/kg; IP; 15-min pretreatment time).

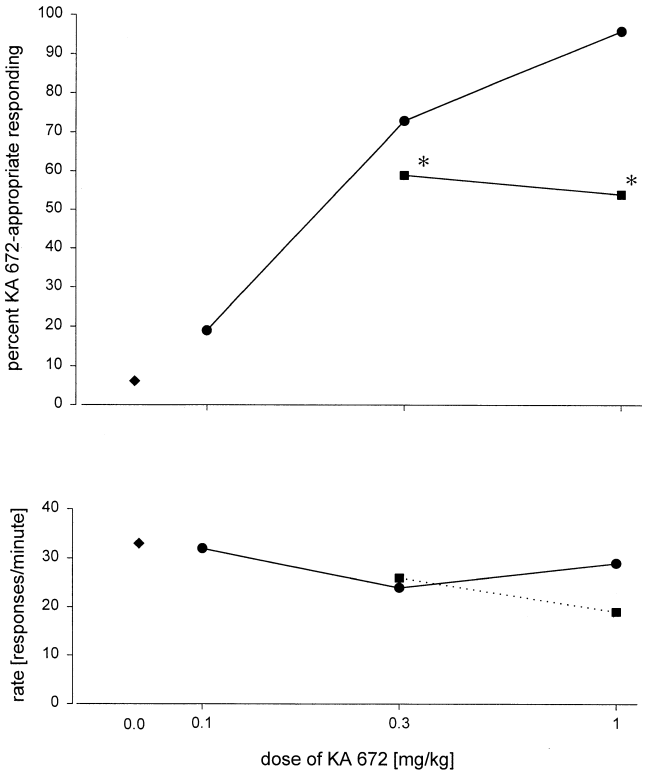


FIG. 2. Dose–response relationship for KA 672 and antagonism by WAY-100635 in rats trained with KA 672 (1.0 mg/kg, IP) as a discriminative stimulus. Each point is the mean of one determination in each of five subjects. Circles represent the effects of KA 672 alone, and squares the effects of KA 672 following the administration (SC; 0.3 mg/kg) of WAY-100635, 60 min before testing. The diamond at the 0.0 dose indicates the effects of WAY-100635 alone. Ordinate: upper panel—mean percentage of responses on the KA 672-appropriate lever; lower panel—response rate. Abscissa: dose plotted on a log scale. *Significantly different from both training conditions.

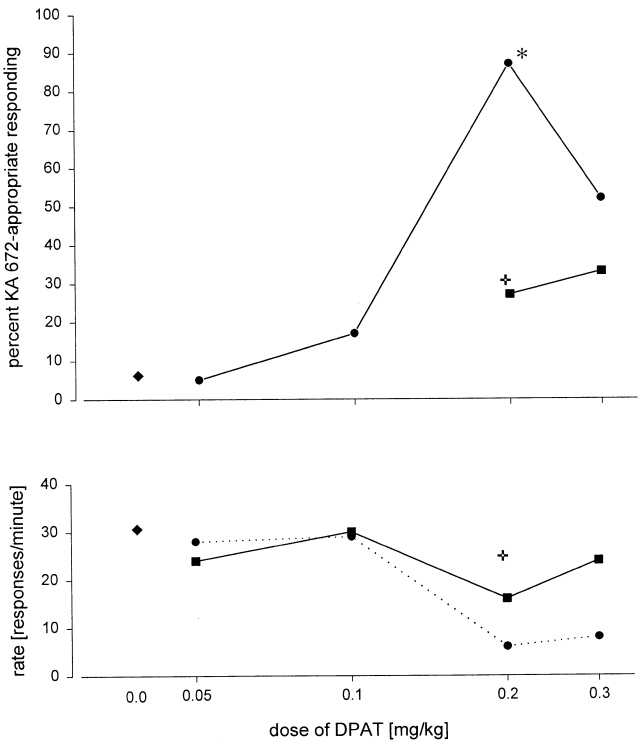


FIG. 4. Dose–response relationship for 8-OH-DPAT and antagonism by WAY-100635 in rats trained with KA 672 as a discriminative stimulus. Circles represent the effects of 8-OH-DPAT alone and squares the effects of 8-OH-DPAT following the administration of WAY-100635. The diamond at the 0.0 dose indicates the effects of the vehicle training condition. *Significantly different from vehicle but not significantly different from KA 672. +Significantly different from 8-OH-DPAT alone. All other details are as in Fig. 2.

TABLE 1

EFFECTS OF SELECTED DRUGS IN RATS TRAINED WITH KA 672 AS A DISCRIMINATIVE STIMULUS

Test Drug	Pretreatment (min)	Dose (mg/kg)	n/N*	KA 672-Appropriate Responding (Percent)	Rate†
Scoparone	15	1	4/4	0	28
		3	4/4	9	31
		10	2/4	29	14
CD-127	15	1	4/4	0	32
		3	4/4	0	28
		10	4/4	9	11
OMPP	15	1	4/4	0	21
		10	1/4	0	5
Ketamine	30	3	4/4	10	34
		10	4/4	41‡	22
		20	0/4	—	0
PCP	30	2	5/5	20‡	31
		3	2/5	17	8
Dizocilpine	60	0.05	5/5	46‡	21
		0.1	4/6	42‡	17
		0.2	3/7	85§	6
Urapidil	15	1	5/5	28	26
		3	7/7	59‡	11
		10	5/5	45	9
Apomorphine	15	0.3	5/5	38‡	33
		0.6	4/6	49	16
		1	2/3	28	8
		3	2/6	38	6

*n = number of animals completing the test; N = number of animals tested.
†Response rate expressed as responses per minute.
‡Significantly different from both training conditions.
§Significantly different from saline but not from KA 672.

subjects. Intermediate generalization, i.e., a degree of KA 672-appropriate responding significantly different from both the saline and KA 672 training conditions, was observed following one or more doses of ketamine, PCP, dizocilpine, urapidil, and apomorphine.

DISCUSSION

The present results indicate that KA 672 is efficacious as a discriminative stimulus and that, using a 15-min pretretment time, its stimulus effects are completely absent after 2 h. The absence of significant generalization either to ortho-methoxyphenylpiperazine, one-half of the KA 672 structure, or to the coumarin derivatives, CD-127 and scoparone, which represent the other half of KA 672, suggests that both moieties represent structural features essential for its stimulus properties.

Because of the purported efficacy of KA 672 as a cognitive enhancer (12), its high-affinity for 5-HT_{1A} receptors (16), and the possible role played by those receptors in memory (6,11, 21), it was of interest to assess its interactions with the 5-HT_{1A}-selective drugs, 8-OH-DPAT and WAY- 100635. Evidence for a 5-HT_{1A}-mediated component in KA 672-induced stimulus control is provided by (a) antagonism of the KA 672 cue by WAY-100635 (Fig. 2), and (b) full generalization of KA 672 to 8-OH-DPAT with blockade of that generalization by WAY-100635 (Fig. 4). However, the fact that WAY-100635 did not completely antagonize KA 672-induced stimulus control suggests the presence of stimulus elements mediated by receptors other than that of the 5-HT_{1A} type.

The remainder of the drugs listed in Table 1 were chosen to provide a preliminary assessment of the possible role in KA 672-induced stimulus control of effects mediated by NMDA/PCP receptors (ketamine, PCP, dizocilpine), dopaminergic receptors (apomorphine), and adrenergic receptors (urapidil). Although a statistically significant intermediate degree of generalization to both PCP and ketamine was observed, the pharmacological significance of this effect is uncertain; in both cases the magnitude of the effect was modest and higher doses could not be explored because of rate suppression. In contrast, full generalization was observed to dizocilpine, a noncompetitive NMDA antagonist that has been reported both to impair and to enhance memory (13–15). This observation is particularly interesting in light of a recent report that KA 672 antagonizes NMDA-induced convulsions in mice (5). Significant effects were also seen for individual doses of urapidil and apomorphine; in both cases the degree of generalization was intermediate in nature, i.e., different from both training conditions. The results with urapidil are particularly interesting given the presence of the ortho-methoxy-phenylpiperazine moiety and the drug's activity both as an $alpha_1$-adrenoceptor antagonist (18) and a partial agonist at 5-HT_{1A} receptors (10). KA 672 failed to generalize (data not shown) to the $alpha_1$-adrenoceptor antagonist, prazosin, the sigma receptor ligand, DTG, or the 5-$HT_{1A,2A}$ agonist, 5-MeO-DMT.

The present data demonstrate the efficacy of KA 672 as a discriminative stimulus. However, definitive conclusions cannot be drawn regarding the pharmacological mechanisms that mediate KA 672-induced stimulus control. The strongest indi-

cation, based upon generalization to 8-OH-DPAT and partial antagonism by WAY-100635, is that the 5-HT_{1A} receptor plays a significant role. In addition, the data are suggestive of a functionally significant interaction with receptors of the PCP/NMDA types. It is of interest, given the purported activity of KA 672 as a cognitive enhancer, that receptors of both types have been implicated in the processes of learning and memory.

ACKNOWLEDGEMENTS

All animals used were maintained in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals [Publication No. 85-23, revised 1985]. All experimental protocols were approved by the Laboratory Animal Care Committee of SUNY at Buffalo. This study was supported in part by Dr. Willmar Schwabe GmbH & Co., Karlsruhe, Germany. We thank Ms. Deborah Petti and Ms. Barbara Winter for technical contributions.

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