Pharmacology Biochemistry & Behavior, Vol 18, pp 395–400, 1983

Effects of Ketocyclazocine Alone and in Combination with Naloxone on Schedule-Controlled Responding in Squirrel Monkeys[1]

KATHARINE S MILAR[2] AND LINDA A DYKSTRA[3]

Department of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

Received 13 September 1982

MILAR K S AND L A DYKSTRA *Effects of ketocyclazocine alone and in combination with naloxone on schedule-controlled responding in squirrel monkeys* PHARMACOL BIOCHEM BEHAV **18**(3) 395–400, 1983 —A multiple, fixed interval 5 minutes, fixed ratio 30, schedule of food presentation (Mult FI FR) was used to evaluate the effects of ketocyclazocine, a κ-receptor agonist, in four squirrel monkeys Two monkeys were initially trained with 1-minute time-out (TO) periods between the components of the multiple schedule and two monkeys were initially trained without these TO periods Ketocyclazocine dose-response functions were determined for each monkey under their original training conditions and then the conditions were reversed and dose-response functions were re-determined under the new conditions Ketocyclazocine consistently decreased rates of responding during the FR component of the multiple schedule under both TO and no TO conditions Under the FI component, ketocyclazocine's effects differed dependent upon dose, conditioning history, and the presence or absence of TO periods Intermediate doses of ketocyclazocine (0 01–0 056 mg/kg) increased FI rates of responding under the no TO condition in monkeys originally trained under this condition, however, ketocyclazocine did not increase FI rates of responding under the no TO condition in monkeys originally trained under the TO condition Under the TO condition, intermediate doses of ketocyclazocine did not increase FI rates of responding High doses of ketocyclazocine (0 1 and 0 17 mg/kg) decreased FI rates of responding in all monkeys under both the TO and no TO conditions Naloxone, in doses up to those which decreased responding when given alone, failed to antagonize completely the rate decreasing effects of ketocyclazocine

Ketocyclazocine Schedule-controlled behavior Naloxone antagonism Opiate receptors

SINCE the work by Martin and his associates [4,12] suggesting the existence of three separate opiate receptors—μ, κ, and σ—there have been a number of investigations designed to characterize these receptors physiologically, pharmacologically and behaviorally In these studies, morphine has been examined as the prototype agonist for the μ receptor, ketocyclazocine for the κ receptor and SKF 10047 for the σ receptor [7,9]

The effects of ketocyclazocine can be distinguished from those of morphine in a variety of ways For example, although ketocyclazocine shares the analgesic properties of morphine in rodents [2], it does not produce the characteristic Straub tail or running in mice seen after morphine administration [9,12] Ketocyclazocine neither suppresses nor precipitates morphine withdrawal in morphine-dependent monkeys [21] or chronic spinal dogs [12] It does not produce physical dependence, nor is it self-administered by rhesus monkeys [21], moreover, monkeys tolerant to ketocyclazocine do not show cross tolerance to morphine nor do morphine tolerant monkeys show cross tolerance to ketocyclazocine [11]

Squirrel monkeys and rats trained to respond differentially in the presence of morphine and its injection vehicle do not respond to ketocyclazocine as if it were morphine [15,17] In contrast, pigeons similarly trained do respond to ketocyclazocine as if it were morphine [8] Similarities between the effects of ketocyclazocine and morphine on responding under operant schedules of food presentation have been observed in rats [7] and in rhesus monkeys [11] It has been suggested that the agonists at the different opiate receptors may be distinguished on the basis of naloxone or naltrexone antagonism [7] For example, whereas naloxone antagonizes the effects of both morphine (μ agonist) and ketocyclazocine (κ agonist) in rats responding under a schedule of food presentation, the dose of naloxone required to antagonize ketocyclazocine effects is much higher than

[1]This work was supported by U S Public Health Service Grants GM-22683 and MH-14269

[2]Current address Department of Psychology, Earlham College, Richmond, IN 47374

[3]Recipient of Research Scientist Development Award DA-00033 Requests for reprints should be addressed to Linda A Dykstra, Department of Psychology Davie Hall 013A, University of North Carolina, Chapel Hill, NC 27514

the dose required to antagonize morphine's effects Further, the effects of SKF 10047 (σ agonist) are not antagonized by naloxone [7] In rhesus monkeys, the weakness and sedation produced by ketocyclazocine is only partially and temporarily reversed by naloxone [21] In addition, although both ketocyclazocine and morphine decrease rates of responding under a multiple schedule of food presentation in rhesus monkeys, ketocyclazocine effects are more difficult to reverse with naloxone than are morphine's [11] In the guinea pig ileum, naloxone is also less potent in antagonizing the effects of ketocyclazocine than the effects of morphine [10]

We were interested in assessing the effects of ketocyclazocine on schedule controlled performance in squirrel monkeys and determining whether its effects on the characteristic rates and patterns of responding produced by schedules of reinforcement resemble the effects of morphine The effects of morphine in squirrel monkeys responding under a multiple fixed interval (FI) 10-min, fixed ratio (FR) 30 schedule of food presentation have been examined previously [5] In this situation, morphine generally decreased rates of responding under both FI and FR components The rate decreasing effects of morphine were antagonized by naloxone

In the present study the effects of ketocyclazocine are examined in squirrel monkeys responding under a multiple fixed interval, fixed ratio schedule of food presentation In addition, naloxone antagonism of ketocyclazocine effects on responding are investigated

METHOD

Animals

Four adult male squirrel monkeys (*Saimiri sciureus*) were reduced to 80% of their free-feeding weights Monkeys were housed individually and given Vitamin C either in tablet form or as fresh fruit, daily in conjunction with Purina Monkey Chow sufficient to maintain them at 80% ad lib weight Water was continuously available in the home cage Monkeys S-853 and S-856 had previously received meperidine and naloxone under a multiple schedule of food reinforcement Monkeys S-2 and S-8 had previously received a variety of pharmacological agents under a shock titration schedule

Apparatus

Experiments were conducted with the monkey restrained by a waist lock in the seated position in a small primate cockpit The cockpit was enclosed in a ventilated, sound-attenuated chamber equipped with continuous white masking noise The front panel of the chamber, facing the monkey, contained a centrally located recessed key light and a response lever (BRS/LVE 121-05) mounted 8 5 cm below the key and which required a force of 25–30 g to operate A food cup was located directly below the lever for delivery of 190 mg Noyes banana pellets Electromechanical programming and recording equipment were located in an adjacent room

Procedure

Monkeys performed under a multiple fixed ratio, fixed interval schedule (Mult FR FI) of food presentation Each session began with a FI component signalled by a red stimulus light The first response that occurred after 5 minutes had elapsed produced a banana pellet and initiated the FR component which was signalled by a white stimulus light In the FR component the 30th response produced a food pellet and initiated the next FI component If the monkey did not make 30 responses within 90 sec during the FR component, the schedule changed to the FI component If the monkey did not respond within 90 sec after 5 min had elapsed during the FI component, the schedule changed to the FR component The experimental session consisted of 24 components, 12 FI and 12 FR in alternation Under a second condition, the same multiple schedule of food presentation was employed, except that 1-min time-out (TO) periods separated the components During the TO periods, the chamber was dark and responding had no consequences The TO manipulation was introduced after our obtained FR rates of responding were found to be lower than those obtained by other investigators [5]

S-853 and S-856 were trained initially under the multiple schedule without TO periods and S-2 and S-8 were trained initially under the multiple schedule with TO periods Ketocyclazocine dose-response functions were determined for each monkey under the original training conditions and then the conditions were reversed and ketocyclazocine dose-response functions were re-determined under the new conditions S-2 died from causes unrelated to the drug regimen prior to completion of the dose-response curve under the second multiple schedule condition (no TO), however available drug data are reported After dose-response curves had been obtained for ketocyclazocine alone under the multiple schedule with time out, the effects of selected doses of ketocyclazocine were re-determined in the presence of naloxone

Pharmacological Procedure

A stock solution of 0 3 mg/cc of ketocyclazocine was made by dissolving the drug in 8 5% lactic acid and sonicating the solution in a warm water bath One N NaOH was added to adjust the pH to approximately four The ratio of lactic acid to NaOH was 3 2 in the vehicle Doses of 0 17, 0 10, 0 056, 0 03, 0 01 0 003, and 0 001 mg/cc were made by successive dilutions of the stock solution with the vehicle Ketocyclazocine was administered intramuscularly in the thigh 10 min prior to the experimental session in an injection volume of 1 cc/kg body weight Naloxone HCl was dissolved in distilled de-ionized water also for an injection volume of 1 cc/kg On days for which double injections were scheduled, one injection was given in each thigh 10 min prior to the session Drugs were administered in ascending or descending order on Tuesdays and Fridays with Thursdays serving as a non-injection control day Unless otherwise specified, each monkey received at least two administrations of each dose and 3 to 5 vehicle injections during each behavioral procedure

Data Analysis

Overall rates of responding during the experimental session were calculated separately for fixed ratio and fixed interval components Performance under drug conditions was expressed as a percent of response rate on the preceding non-injection control day In addition, the number of responses that occurred in successive 30-sec intervals or "bins" of the FI 5 min were cumulated over the session and rates of responding in each bin were calculated for each session

RESULTS

Table 1 presents the rates of responding for individual monkeys under each component of the multiple schedule for both experimental procedures The addition of TO periods between the FI and FR components resulted in higher rates of responding during the FR for all monkeys Three of the four monkeys responded at lower rates during the FI component under the TO procedure than under the no TO procedure

Ketocyclazocine dose-effect curves for responding under the multiple FI 5-min, FR 30 schedules (with and without TO between components) are shown for individual monkeys in Fig 1 Vehicle injections produced little or no change in responding under either of the multiple schedule conditions Ketocyclazocine produced a dose related decrease in FR rates of responding under both the TO and no TO conditions Generally, higher doses of ketocyclazocine were required to decrease fixed ratio rates of responding when a TO was between components For example, responding during the FR component was reduced to less than 12% of control rates of responding following 0 1 mg/kg of ketocyclazocine in the no TO condition for all monkeys Comparable rate decreases occurred only following 0 17 mg/kg of ketocyclazocine under the TO condition (see Fig 1)

The effects of ketocyclazocine on rates of responding during the FI component depended on whether or not the TO condition was in effect and whether the monkeys had been examined under the TO condition first or second In monkeys S-853 and S-856, which were initially trained without a TO between components of the multiple schedule, ketocyclazocine increased FI rates of responding at doses between 0 01 and 0 056 mg/kg Rate increases ranged from 30 to 100% of control rates of responding When the dose-effect curve was re-determined in these monkeys with a TO between components, ketocyclazocine did not increase FI rates of responding In monkeys S-2 and S-8, which were initially trained with a TO between components of the multiple schedule, ketocyclazocine generally decreased rates of responding under both multiple schedule conditions At very low doses (0 003 and 0 01 mg/kg) ketocyclazocine increased FI rates of responding only under the TO condition The maximum rate-increasing effect observed, however, was a 14% increase in responding for monkey S-8 at 0 003 mg/kg Rate increases were not observed under the no TO condition for either S-2 or S-8 In addition, rates of responding during the FI component were decreased by lower doses of ketocyclazocine under the multiple schedule with no TO than with TO (see Fig 1)

Ketocyclazocine-induced increases in rate of responding under the no TO condition were further investigated by examining response rates in each 30-sec bin of the FI component following vehicle and a dose of 0 03 mg/kg ketocyclazocine This dose of ketocyclazocine increased rates of responding in the monkeys which were initially trained under the no TO condition, but did not increase rates of responding in monkeys which were initially trained under the TO condition All monkeys showed the typical FI pattern of responding following vehicle injections, i e , rates were low early in the interval and gradually increased later in the interval In those monkeys (S-2 and S-8) for which ketocyclazocine did not increase rates of responding, the high rates of responding which occurred at the end of the fixed interval were decreased by the drug In those monkeys (S-853 and S-856) for which ketocyclazocine increased rates of responding during the FI component, the drug-induced increases were primarily due to initiating responding earlier in the interval and responding at a slightly higher rate throughout the interval

TABLE 1

MEAN RESPONSES/SEC (±S E) DURING FI AND FR COMPONENTS OF MULTIPLE SCHEDULES WITH TIME OUT (TO) AND WITHOUT TIME OUT (no TO) FOR INDIVIDUAL MONKEYS

		FR	FI
S-853	no TO	0 722 ± 0 049	0 071 ± 0 008
	TO	1 52 ± 0 08	0 049 ± 0 004
S-856	no TO	0 943 ± 0 035	0 312 ± 0 019
	TO	1 77 ± 0 126	0 272 ± 0 018
S-2	no TO	1 13 ± 0 046	0 269 ± 0 01
	TO	1 57 ± 0 059	0 247 ± 0 011
S-8	no TO	1 62 ± 0 049	0 896 ± 0 03
	TO	2 33 ± 0 059	1 22 ± 0 043

Figure 2 shows the effects of the two highest doses of ketocyclazocine alone and in combination with two doses of naloxone under the TO condition for all monkeys The 1 0 mg/kg dose of naloxone had no effect on FI rates of responding but decreased FR rates of responding slightly In contrast, 3 0 mg/kg of naloxone produced a rate decrease of approximately 30% for both FR and FI response rates when administered alone A dose of 1 0 mg/kg naloxone effectively antagonized the rate-decreasing effects of 0 1 mg/kg ketocyclazocine but did not antagonize the effects of 0 17 mg/kg ketocyclazocine The 3 0 mg/kg dose of naloxone did not antagonize the effects of either dose of ketocyclazocine

DISCUSSION

Ketocyclazocine consistently decreased rates of responding under the FR component of a multiple schedule of food presentation under both TO and no TO conditions Under the FI component, ketocyclazocine's effects differed dependent upon conditioning history and the presence or absence of a TO between schedule components Intermediate doses of ketocyclazocine increased FI rates of responding under the no TO condition in monkeys initially trained under this condition, however, in monkeys initially trained under the TO condition and then re-trained under the no TO condition, these doses of ketocyclazocine did not increase FI rates of responding

The effects of ketocyclazocine under the TO condition resemble the effects of morphine in squirrel monkeys responding under a multiple FI 10-min FR 30 schedule of food presentation with 1-min TO periods between components in that both morphine and ketocyclazocine produced dose-related decreases in rates of responding during both components of the multiple schedule [5] In the Goldberg study, a single low dose of morphine increased rates of responding under the FI component by approximately 40% in 2 of the 8 monkeys examined In the present study, low doses of ketocyclazocine only slightly increased rates of FI responding in the two monkeys originally trained under the TO condition The effects of ketocyclazocine and morphine on rates of responding during the FI component were different for

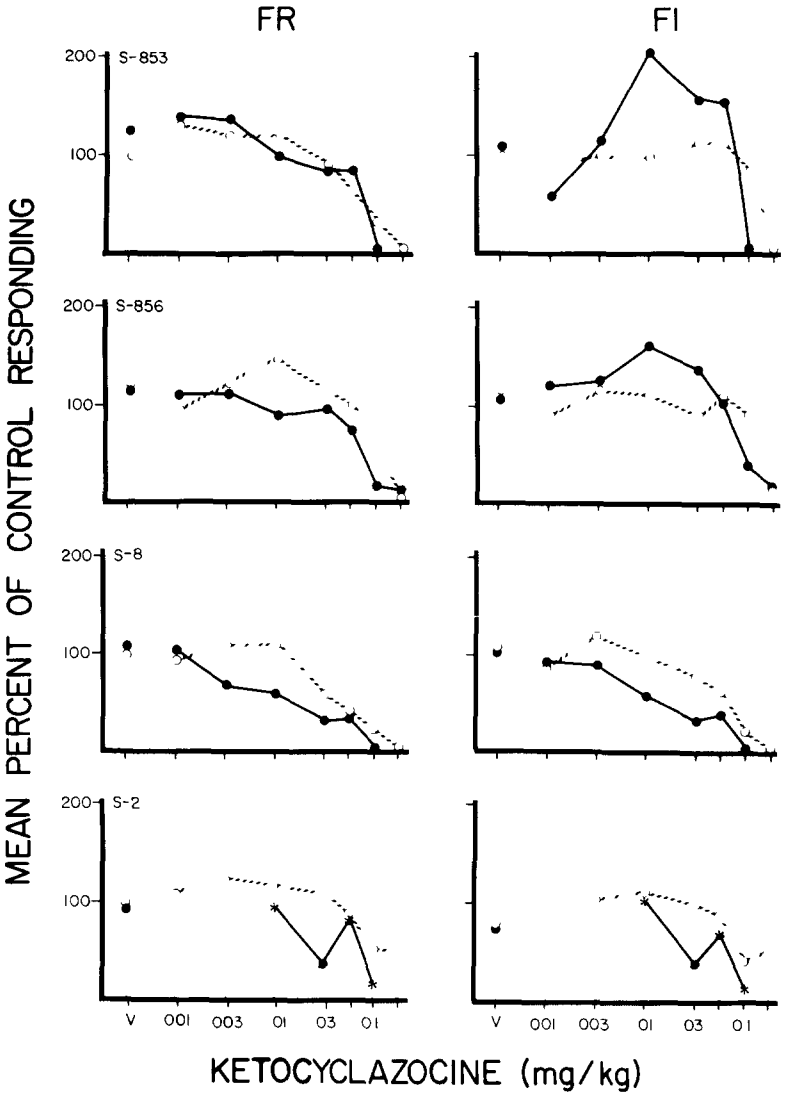

FIG 1 Mean percent of control responding for FR and FI components as a function of ketocyclazocine dose and vehicle injections (V) in individual monkeys Closed circles represent performance under the no TO condition and open circles represent performance under the TO condition S-853 and S-856 were initially trained under the no TO condition S-2 and S-8 under the TO condition Asterisks indicate only 1 determination obtained at that dose

monkeys originally trained under the no TO condition, however Ketocyclazocine markedly increased FI rates of responding over a broad dose range in the no TO condition for the 2 monkeys originally trained under this condition

It may be that the ketocyclazocine-induced rate increases in the no TO condition for monkeys originally trained under this condition are a result of an interaction between the FR and FI components The abrupt transition from the reinforcement rich FR schedule to the relatively lean FI schedule may produce a type of response suppression that contributes to the pause at the beginning of the fixed interval component If ketocyclazocine eliminates this suppression, the pause would be shortened and response rate would be increased The addition of a TO between components should dissipate this schedule interaction Unfortunately, no data on pause length were collected, although the rate by FI bin analysis did show that doses of ketocyclazocine which increased rates of responding, decreased pause length

Although ketocyclazocine increased responding under the no TO condition for monkeys initially trained under this condition, it did not increase FI rates of responding under the no TO condition in monkeys initially trained under the TO condition and re-trained under the no TO condition Schedule interaction effects in the no TO condition could have been eliminated by the TO conditioning history Alternatively, greater experience with the no TO condition may be necessary before rate increases are observed The two monkeys originally trained with no TO had an extensive his-

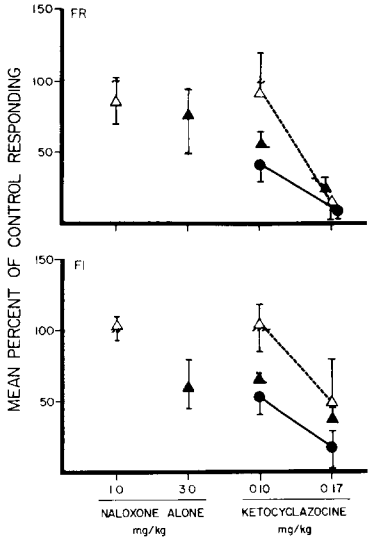

FIG 2 Mean percent of control responding (±S E) in FR and FI components for four monkeys under the TO condition Open triangles represent 1 0 mg/kg of naloxone alone and in combination with ketocyclazocine, closed triangles represent 3 0 mg/kg of naloxone alone and in combination with ketocyclazocine Closed circles represent ketocyclazocine alone

tory of responding on this schedule Although drugs were not administered to monkeys originally trained with TO until responding had stabilized under the no TO condition, their experimental history with no TO was considerably less than the other two monkeys In rats, conditioning history has been shown to influence control rates of responding under an FI schedule of food presentation as well as the effects of *d*-amphetamine [18] Interestingly, conditioning history did not alter control rates nor drug effects in rats responding under a VI schedule of food presentation [14] Conditioning history has also been shown to alter drug response without concomitant alteration in control rates of responding in squirrel monkeys responding under punishment [1] and escape schedules [16] Further experiments with a larger number of subjects in each sub-group are necessary to validate this observation on the influence of conditioning history and the observed rate increases on FI responding

Ketocyclazocine and morphine exert similar effects under some behavioral conditions (e g , [7,11]) and different effects under other behavioral conditions (e g , [19,20]) making it difficult to distinguish different opiate receptor activity on the basis of drug-behavior interactions alone Difference in receptor activity are implicated however by the fact that the effects of morphine are more readily antagonized by naloxone than are the effects of ketocyclazocine For example, in squirrel monkeys responding under a multiple schedule of food presentation, 0 1 mg/kg naloxone completely antagonized decreased rates of responding when responding had been reduced to less than 20% of control rates by morphine administration [5] In the present study, naloxone did not antagonize the effects of a dose of ketocyclazocine which decreased rates of responding to less than 20% of control although antagonism of a lower dose of the drug was obtained Similarly, the dose of naloxone required to antagonize the effects of ketocyclazocine in rats responding under a FI 5 min schedule of food presentation, was much higher than the dose required to antagonize morphine's effects [7] Further, Llewellyn [11] found that the effects of ketocyclazocine in rhesus monkeys responding under a multiple schedule of food presentation were more difficult to antagonize with naloxone than were similar effects of morphine

In summary, ketocyclazocine's effects on responding under a multiple schedule of food presentation resembled the effects of morphine under the FR component of this schedule Under the FI component, comparisons between ketocyclazocine and morphine were more difficult to make since ketocyclazocine's effects differed dependent on conditioning history Nevertheless, naloxone was clearly less potent in antagonizing ketocyclazocine's effects than it was in antagonizing morphine's effects under similar conditions [5]

ACKNOWLEDGEMENTS

We thank Sam Lerner for his help in collecting the data Jeff Witkin and Bob MacPhail for their comments on previous versions of the manuscript and J D Leander for his suggestions and the ketocyclazocine

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