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EFFECTS OF 1-METHYL-1,2,3,4-TETRAHYDROISOQUINOLINE ON THE BEHAVIORAL EFFECTS OF COCAINE IN RATS

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The efficacy of 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ), a member of endogenous tetrahydroisoquinolines, in cocaine- and food-maintained responding in self-administration procedures under a fixed ratio 5 schedule of reinforcement as well as in cocaine and food seeking behaviors in male Wistar rats was examined. The effects of 1MeTIQ on cocaine discrimination and on basal locomotor activity were also assessed. In rats trained to self-administered either cocaine (0.5 mg/kg/injection) paired with the cue (light+tone) or food under a fixed ratio 5 schedule of reinforcement, 1MeTIQ (25 - 50 mg/kg) dose-dependently decreased the cocaine-maintained responding, but did not alter the food-maintained responding. 1MeTIQ (25 - 50 mg/kg) decreased the cocaine seeking behavior reinstated by a noncontingent presentation of cocaine (10 mg/kg, *i.p.*), but altered neither behavior reinstated by a discrete cue (tone+light) nor food-induced reinstatement. In rats trained to discriminate cocaine (10 mg/kg) from saline in water-reinforced fixed ratio 20 task, pretreatment with 1MeTIQ resulted in neither substitution nor significant alterations in the cocaine (1.25 - 10 mg/kg)-induced discriminative stimulus effects. 1MeTIQ (25 - 50 mg/kg) did not produce also a significant changes in basal horizontal activity. In conclusion, our present results outline a significance of exogenously applied 1MeTIQ in attenuating drug-evoked relapses to cocaine as well as the direct rewarding properties of cocaine (that model the cocaine-induced "high"), but not cocaine subjective effects. Moreover, a dissociation between effects of 1MeTIQ on cocaine vs. food-maintained responding was demonstrated.

Key words: *1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ), cocaine, drug discrimination, food, locomotor activity, self-administration*

INTRODUCTION

Cocaine addiction is a life-threatening disease for which there is currently no effective pharmacotheraphy (1). Both clinical and preclinical findings indicate that the behavioral response to cocaine, including discriminative stimulus and rewarding effects as well as reinstatement of cocaine seeking behavior, depend on the drug ability to block the dopamine transporter, thereby increasing dopamine extracellular concentration within the mesocorticolimbic system and indirect activation of dopamine D1 and D2 receptors (for review see: 2 - 5).

Confirming further the role of dopamine neurotransmission in cocaine behaviors we have recently demonstrated that 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ) prevents the expression of the behavioral and neurochemical consequences of cocaine-primed induced reinstatement (6). 1MeTIQ is a member of endogenous tetrahydroisoquinolines (7, 8) and shows neuroprotective properties depending on alterations in both the dopaminergic (9, 10) and glutamatergic neurotransmitter systems (11). In fact, 1MeTIQ inhibits the MAO-dependent oxidative deamination of dopamine and shifts catabolism of dopamine towards COMT-dependent O-methylation (12). In the *in vitro* binding assays, 1MeTIQ displaced the non-selective dopamine agonist [^{3}H]apomorphine with a potency comparable to that of the endogenous dopamine, but not the dopamine antagonist [^{3}H]spiperone what indicates its direct interaction with the agonistic (active) conformation of dopamine receptors (6). On the other hand, in the *in vivo* studies it shows antidopaminergic activity (12). 1MeTIQ was also shown to prevent the glutamate-induced cell death in granular cell cultures and in the *in vivo* microdialysis it decreased the release of excitatory amino acids evoked by intra-prefrontal cortex kainate injection in the rat (13). Additionally, 1MeTIQ displays an affinity for the NMDA glutamatergic receptors as confirmed by the inhibition of [^{3}H]MK-801 binding (13).

In the present study we used self-administration and drug discrimination procedures to test the hypothesis that 1MeTIQ would decrease rewarding and stimulus effects of cocaine in rats. Additionally, we investigated the effects of 1MeTIQ on the cocaine-induced or the conditioned cue-induced reinstatement of drug-seeking behavior following chronic intravenous cocaine self-administration in an animal model of relapse. Finally, we examined the effects of 1MeTIQ on the maintenance of food self-administration and on the reinstatement of responding induced by food as well as on basal locomotor activity.

MATERIALS AND METHODS

Animals

The experiments were performed on male Wistar rats (10 - 12 weeks of age) derived from the licenced breeder (T. Górzkowska, Warszawa, Poland). The rats were housed individually (self-

administration procedures), 2/cage (drug discrimination), or 8/cage (locomotor activity) in standard plastic rodent cages in a colony room maintained at 20 ± 1°C and at 40-50% humidity under a 12 h light-dark cycle (lights on at 06:00). Animals had free access to food (Labofeed pellets) and water during the 7-day habituation period. Then, rats used in locomotor activity studies had free access to water, while those used in the cocaine self-administration procedures were maintained on limited water during initial training sessions (see below), or the amount of water that an animal received was restricted to that given during daily training sessions (5 - 6 ml/rat/session), after test sessions (15 min) and on weekends (36 h) in drug discrimination procedures (see below), while animals used in the food self-administration procedures were maintained on limited food intake (see below). All experiments were conducted during the light phase of the light-dark cycle (between 08:00 - 15:00) and were carried out in accordance with the National Institutes of *Health Guide for the Care and Use of Laboratory Animals* and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 1997). The animals were experimentally naive.

Drugs

The following drugs were used: 1MeTIQ (1-methyl-1,2,3,4-tetrahydroisoquinoline; synthesized by Dr. J. Boksa, Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland) and cocaine hydrochloride (Sigma-Aldrich, USA). 1MeTIQ was injected intraperitoneally (*i.p.*) in a volume of 1 ml/kg, while cocaine was given either intravenously (*i.v.*) in a volume of 0.1 ml/infusion or *i.p.* in a volume of 1 ml/kg. 1MeTIQ and cocaine were dissolved in sterile 0.9% NaCl. 1MeTIQ was administered 30 min, while cocaine (*i.p.*) either 15 min (drug discrimination procedures) or immediately (reinstatement of seeking behavior) before tests. The doses and pretreatment time of drugs were chosen based upon its activity in previous *in vivo* studies (9, 10, 12, 13).

Cocaine self-administration

Maintenance: Rats were trained to press the lever of standard operant conditioning chambers (Med-Associates, USA) under a fixed ratio 5 schedule of water reinforcement. Two days following "lever-press" training and free access to water, the rats were chronically implanted with a silastic catheter in the external right jugular vein, as described previously (14). Catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml, Biochemie, Austria) and 0.1 ml of solution of cephazolin (10 mg/ml; Biochemie GmbH, Austria). Catheter patency was tested periodically with the ultrashort-acting barbiturate anaesthetic methohexital (10 mg/kg, *i.v.*; loss of consciousness within 5 s). After a 10-day recovery period, all animals were water deprived for 18 h and trained to lever press to fixed ratio 5 schedule of water reinforcement over a 2-h session. Subjects were then given access to cocaine during 2-h daily sessions performed 6 days/week and from that time they were given *ad libitum* water. The house light was illuminated throughout each session. Each completion of five presses on the "active" lever complex (fixed ratio 5 schedule) resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and a 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the "active" lever and the tone generator, 2000 Hz; 15 dB above ambient noise levels). Following each injection, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the conditioned operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 18 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of <10% of the average; this criterion was selected based on our prior experiments (14). Once stable rates of responding were established, rats (n = 7) were pretreated with either vehicle or 1MeTIQ (25 - 50 mg/kg) before the test sessions which were

separated by at least two-three baseline days of cocaine self-administration. The order of injections was counterbalanced according to a Latin square design, and test sessions were separated by at least two-three baseline days of a dose unit cocaine self-administration.

Cocaine priming-induced reinstatement: A separate group of rats ($n = 8$) was tested for response reinstatement induced by a noncontingent presentation of the cocaine priming (10 mg/kg, *i.p.*). Animals were trained to self-administered cocaine as described above. Acquisition of the conditioned operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 18 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of <10% of the average; this criterion was selected based on our prior experiments (14). Once stable rates of responding were established, the extinction procedure was carried out on the following day. During extinctions sessions subjects had 2-h daily training sessions with no delivery of cocaine or the presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below 10% of the level observed during maintenance during at least 3 consecutive days), the rats were tested for response reinstatement induced by a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, *i.p.*). Before test sessions rats were pretreated with either vehicle or 1MeTIQ (25 - 50 mg/kg). During the reinstatement tests (2-h sessions), active lever presses on the fixed ratio 5 schedule resulted only in an intravenous injection of saline. Drug combinations were given in a randomized order in three reinstatement tests that each rat received and test sessions were separated by at least two-three extinction sessions.

Cocaine-associated cue-induced reinstatement: A separate group of rats ($n = 7$) was tested for response reinstatement induced by discrete cue (tone + light previously associated with cocaine self-administration). Animals were trained to self-administered cocaine as described above. Acquisition of the conditioned operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 18 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of <10% of the average; this criterion was selected based on our prior experiments (15). Once stable rates of responding were established, the extinction procedure was carried out on the following day. During extinctions sessions subjects had 2-h daily training sessions with no delivery of cocaine or the presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below 10% of the level observed during maintenance during at least 3 consecutive days), the rats were tested for response reinstatement induced by discrete cue (tone + light previously paired with cocaine self-administration). Before test sessions rats were pretreated with either vehicle or 1MeTIQ (25 - 50 mg/kg). During the reinstatement tests (2-h sessions), active lever presses on the fixed ratio 5 schedule resulted only in an intravenous injection of saline. Drug combinations were given in a randomized order in three reinstatement tests that each rat received and test sessions were separated by at least two-three extinction sessions.

Food self-administration

Maintenance: Food self-administration was conducted in a manner parallel to cocaine self-administration (15). Food-restricted rats (20 g/rat/day) were trained to press the lever of a standard operant chambers (Med-Associates, USA) under a fixed ratio 5 schedule of reinforcement (each completion of a fixed ratio 5 schedule on the "active" lever resulted in a delivery of the portion of sweetened milk (0.1 ml)) in daily 2-h sessions. Following each reward, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in food delivery. The house light was on throughout each session.

Rats remained in maintenance training until lever pressing stabilized (the number of active lever presses varied by 10% or less over the course of 3 consecutive maintenance days). Once stable rates of responding were established, the rats (n = 5 rats) were pretreated with either vehicle or 1MeTIQ (25 - 50 mg/kg). The order of injections was counterbalanced according to a Latin square design, and test sessions were separated by at least two-three baseline days of food self-administration.

Food-induced reinstatement: A separate group of rats (n = 6) was tested for response reinstatement induced by a contingent presentation of food. Animals were trained to self-administered food as described above. Acquisition of the conditioned operant response lasted until rats met the following criteria: the number of active lever presses varied by 10% or less over the course of 3 consecutive maintenance days; this criterion was selected based on our prior experiments (15). Once stable rates of responding were established, the extinction procedure was carried out on the following day. During extinctions sessions subjects had 2-h daily training sessions with no delivery of food. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below 10% of the level observed during maintenance during at least 3 consecutive days), the rats were tested for response reinstatement induced by contingent presentation of food (each completion of a fixed ratio 5 schedule on the "active" lever resulted in a delivery of the portion of sweetened milk (0.1 ml). Following each reward, there was a 20-s time-out period during which responding was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in food delivery. Before the reinstatement tests (2-h sessions) rats were pretreated with either vehicle or 1MeTIQ (25 - 50 mg/kg). Drug combinations were given in a randomized order in three reinstatement tests that each rat received and test sessions were separated by at least two-three extinction sessions.

Cocaine discrimination

Rats (n = 8) with restricted access to water during the daily training sessions (5 - 6 ml/rat/session), after test sessions (15 min), and over the weekends were trained to discriminate cocaine (10 mg/kg; *i.p.*) from 0.9% NaCl (*i.p.*), as described previously (16). Briefly, cocaine or saline was administered 15 min before daily (Monday-Friday) sessions (15 min), in two-lever standard operant conditioning chambers equipped with a water-filled dispenser mounted equidistant between two response levers on one wall (Med-Associates; USA), under a fixed ratio 20 schedule of continuous water reinforcement (0.1 ml per each completed fixed ratio 20) and depending on the treatment left or right lever became active, until all the animals met the criteria (an individual mean accuracy of at least 80% of correct responses, before the first reinforcer during 10 consecutive sessions). Test sessions were conducted once or twice a week. Cocaine and saline sessions intervened between the test sessions to maintain discrimination accuracy. Only the rats that met 80% performance criterion during the preceding cocaine and saline sessions were used in the tests. After completion of 20 responses to either lever, or after the session time elapsed, a single reinforcer was delivered and the animals were removed from the chamber. In substitution tests, rats were tested with different doses of cocaine (1.25 - 10 mg/kg) or 1MeTIQ (25 - 100 mg/kg). In combination tests, a fixed dose of 1MeTIQ (25 or 50 mg/kg) was given before different doses of cocaine.

Locomotor activity measurements

The spontaneous locomotor activity of rats was recorded for each animal as described previously (17). Briefly, the locomotor activity was measured in Opto-Varimex cages surrounded with a 15 x 15 array of photocell beams located 3 cm from the floor surface (Columbus Instruments, Columbus, USA). Interruptions of these photobeams resulted in horizontal activity defined as distance traveled and expressed in cm. Separate records of horizontal activity were

made by the control software (Columbus Instruments) for subsequent statistical evaluation. Before locomotor activity was recorded, the separate groups of rats (n = 6 - 8 rats/group) were injected with vehicle or 1MeTIQ (25 - 50 mg/kg) in their home cages and at the appropriate time were transferred to the experimental cages. Locomotor activity recording started immediately following rat placement and lasted 2 h.

Statistical analyses

In cocaine and food self-administration procedures, the number of active and inactive lever presses (maintenance and reinstatement phases) and the number of cocaine/food reinforcements (maintenance phase) for a group pretreated with 1MeTIQ was analyzed by an one-way analysis of variance (ANOVA) and where appropriate a *post hoc* Dunnett's test was used to analyze differences between group means.

In drug discrimination studies, accuracy was defined as the percentage of correct responses to total responses before the delivery of the first reinforcer (training sessions) or as the percentage of cocaine-lever responses to total responses upon completion of a fixed ratio 20 on either lever (test sessions). Response rates (responses per second), regarded as a measure of behavioral disruption, were calculated as the total number of responses to either lever before completion of the first fixed ratio 20, divided by the number of seconds required to complete the fixed ratio. Only the data from animals that completed the fixed ratio 20 during the test sessions were used. An one-way ANOVA for repeated measures was used to compare the percentage of drug-lever responding and response rates during the test sessions with the corresponding values of the preceding drug session (substitution tests). Post hoc Dunnett's test was used to analyze differences between group means. In combination experiments, a two-way ANOVA for repeated measures was used to analyze the effects of 1MeTIQ (factor 1) and cocaine dose (factor 2).

In the locomotor activity measurements, the data were analyzed by an one-way ANOVA and where appropriate a *post hoc* Dunnett's test was used to analyze differences between group means.

RESULTS

Cocaine self-administration

Maintenance: Rats showed stable responding on levers during the last 6 self-administration maintenance sessions with an acquisition criterion requiring that the rate of active lever presses varied by less than 10%. The animals had self-administered 18 - 22 infusions of cocaine with the daily mean cocaine intake between 9 - 11 mg/kg. Rats responded significantly more frequently on the active lever than on the inactive lever ($p<0.01$), independently of self-administration test day (*Fig. 1*).

Pretreatment with 1MeTIQ (25 - 50 mg/kg) decreased in a dose-dependent manner the number of active lever presses ($F(2,18) = 4.37$, $p<0.05$) and cocaine ($F(2,18) = 4.49$, $p<0.05$), while the number of inactive lever presses remained unaltered ($F(2,18) = 0.61$). The dose of 50 mg/kg of 1MeTIQ significantly attenuated the cocaine-maintained responding (*Fig. 1*).

Cocaine priming-induced reinstatement: After 10 days of extinction during which the active lever presses resulted in the *i.v.* delivery of saline without the

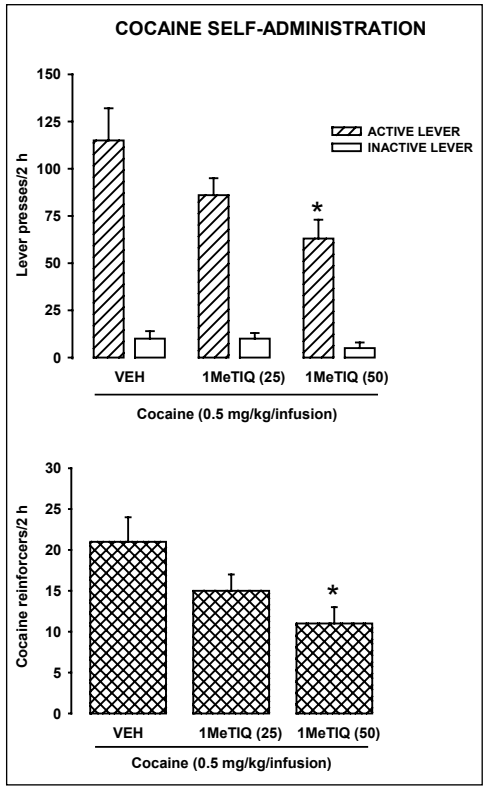

Fig. 1. Effects of 1MeTIQ on cocaine self-administration in rats responding under a fixed ratio 5 schedule of reinforcement. Number of the active (*cross bars*), inactive (*white bars*) lever presses (*upper panel*) and cocaine reinforcers (*cross hatched bars; bottom panel*) during cocaine self-administration following vehicle (VEH) or 1MeTIQ (25 - 50 mg/kg). Self-administration active lever responses resulted in a delivery of a cocaine infusion (0.5 mg/kg per injection) and simultaneous presentation of a light+tone stimulus complex. * $p<0.01$ *vs*. vehicle.

presentation of the conditioned stimulus (cue), the rats were tested for response reinstatement induced by cocaine (10 mg/kg, *i.p.*). During cocaine-induced reinstatement test, the rats responded more frequently on the active lever in relation to the inactive lever ($p<0.05$) and to extinction period ($p<0.05$). The responses on the inactive lever were not different across the test days (*Fig. 2 left panel*).

A significant overall group effect was detected by ANOVA for pretreatment with 1MeTIQ for active ($F(3,28) = 12.1$, $p<0.01$), but not inactive ($F(3,28) = 0.6$) lever presses. 1MeTIQ at 50 mg/kg significantly decreased the response reinstatement on active lever induced by cocaine priming (*Fig. 2 left panel*).

Cocaine-associated cue-induced reinstatement: After 10 days of extinction trials during which active lever presses resulted in the *i.v.* delivery of saline without the presentation of the conditioned stimulus (cue), the rats were tested for response-reinstatement induced by the presentation of the cue associated previously with cocaine infusions. During the cue-induced reinstatement test, the rats responded more frequently on the active lever in relation to the inactive lever ($p<0.05$) and to extinction period ($p<0.05$). The responses on the inactive lever were not different across the test days (*Fig. 2 right panel*).

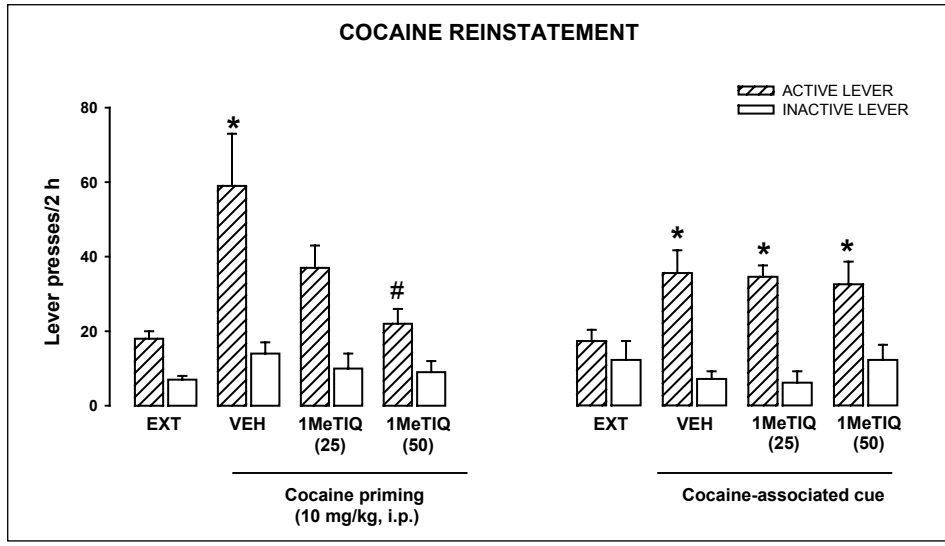

Fig. 2. Effects of 1MeTIQ on reinstatement of cocaine seeking behavior. Number of active (*i.e.* previously associated with cocaine self-administration; *cross bars*) and inactive (*white bars*) lever presses following vehicle (VEH) or 1MeTIQ (25 - 50 mg/kg) in combination with a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, *i.p.*; *left panel*) or a contingent presentation of discrete cues (tone + light previously paired with cocaine self-administration; *right panel*). The baseline extinction (EXT) responding is also presented. * $p<0.01$ *vs*. extinction; # $p<0.05$ *vs*. vehicle.

A significant overall group effect was detected by ANOVA for pretreatment with 1MeTIQ for active ($F(3,24) = 4.14$, $p<0.05$), but not inactive ($F(3,24) = 1.11$) lever presses. 1MeTIQ at doses of 25 or 50 mg/kg failed to alter the response reinstatement induced by the presentation of the cue (*Fig. 2 right panel*).

Food self-administration

Maintenance: Rats showed stable responding on levers during the last 3 self-administration maintenance sessions with an acquisition criterion requiring that the rate of active lever presses varied by less than 10%. Rats responded significantly more frequently on the active lever than on the inactive lever ($p<0.01$), independently of food self-administration test day (*Fig. 3*).

Pretreatment with 1MeTIQ (25 - 50 mg/kg) neither changed the number of active ($F(2,12) = 1.05$) or inactive ($F(2,12) = 1.71$) lever presses nor food reinforcers ($F(2,12) = 1.43$) (*Fig. 3*).

Food-induced reinforcement: After 10 days of extinction the rats were tested for response reinstatement induced by the contingent presentation of food. During the food-induced reinstatement test, the rats responded more frequently on the active lever in relation to the inactive lever ($p<0.001$) and to extinction

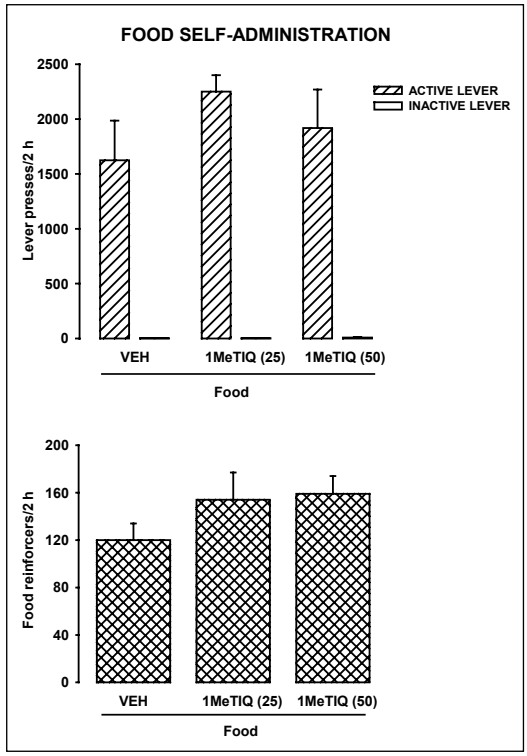

Fig. 3. Effects of 1MeTIQ on food self-administration in rats responding under a fixed ratio 5 schedule of reinforcement. Number of the active (*cross bars*), inactive (*white bars*) lever presses (*upper panel*) and food reinforcers (*cross hatched bars; bottom panel*) during food self-administration following vehicle (VEH) or 1MeTIQ (25 - 50 mg/kg). Self-administration active lever responses resulted in a delivery of food (sweetened milk, 0.1 ml/portion). * $p<0.01$ *vs.* vehicle.

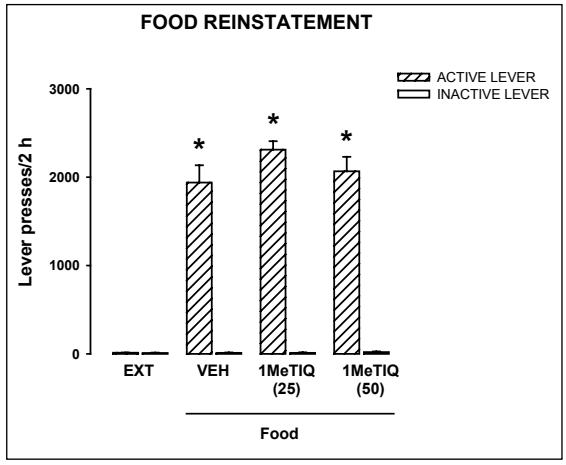

Fig. 4. Effects of 1MeTIQ on reinstatement of food seeking behavior. Number of active (*i.e.* previously associated with food self-administration; *cross bars*) and inactive (*white bars*) lever presses following vehicle (VEH) or 1MeTIQ (25 - 50 mg/kg) in combination with a contingent presentation of food (sweetened milk). The baseline extinction (EXT) responding is also presented. * $p<0.01$ *vs*. extinction.

period ($p<0.001$). The responses on the inactive lever were not different across the test days (*Fig. 4*).

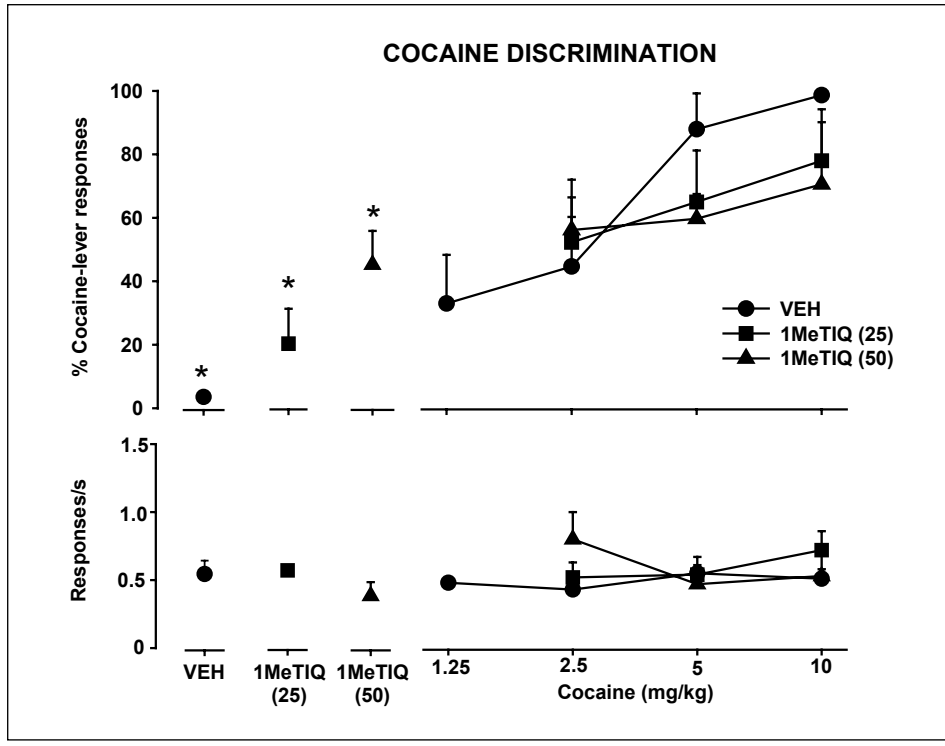

Fig. 5. Effects of 1MeTIQ on cocaine discrimination in rats trained to discriminate cocaine (10 mg/kg) from saline. Data represent the mean percentage of cocaine-lever responses (*upper panels*) and the response rate in mean number of responses/s (*lower panels*). Performance is denoted after injection of vehicle (VEH; circle), 1MeTIQ (25 mg/kg; square), 1MeTIQ (50 mg/kg; triangle), or after administration of cocaine (1.25 - 10 mg/kg) preceded by injection of vehicle (circles) or a fixed dose of 1MeTIQ (25 mg/kg; squares) or 1MeTIQ (50 mg/kg; triangles). * $p<0.01$ *vs*. previous maintenance cocaine session.

A significant overall group effect was detected by ANOVA for pretreatment with 1MeTIQ for active ($F(3,20) = 59.99$, $p<0.001$), but not inactive ($F(3,20) = 0.79$), lever presses. However, the individual dose groups of pretreatment with 1MeTIQ (25 - 50 mg/kg) in combination with food did not indicate a significant alteration in the response reinstatement (*Fig. 4*).

Cocaine discrimination

Acquisition and substitution: The acquisition of cocaine (10 mg/kg) *vs*. saline discrimination was reached in an average of 24 sessions (ranging between: 18 - 29). Administration of cocaine (1.25 - 10 mg/kg) produced a dose-dependent increase in the cocaine-lever responding, while administration of saline evoked less than 5% of drug-lever responding (*Fig. 5 upper panel*). The response rates

after saline and all the test doses of cocaine did not differ from those recorded during the preceding cocaine session (data not shown).

Following administration of 1MeTIQ (25 and 50 mg/kg) weak substitution levels (ca. 20% and 47% cocaine-lever responding, respectively) were found (*Fig. 5 upper panel*). 1MeTIQ at a dose of 100 mg/kg produced a disruption of behavior (0 responses/s) (data nor shown), while at doses of 25 - 50 mg/kg it did not alter animals' responding (*Fig. 5 lower panel*).

Combination studies: There was a main effect of cocaine dose ($F(2,42) = 4.28$, $p<0.02$), but no main effect of 1MeTIQ pretreatment ($F(2,21) = 2.08$) or a 1MeTIQ x cocaine dose interaction ($F(4,42) = 1.14$) as shown by a two-way ANOVA for repeated measures. Pretreatment with 1MeTIQ (25 or 50 mg/kg) did not affect cocaine-lever responding as compared to cocaine given alone, however a trend to attenuate cocaine (5 - 10 mg/kg)-induced discriminative stimulus was observed (*Fig. 5 upper panel*).

There was no main effect of pretreatment with 1MeTIQ ($F(2,21) = 0.6$) or cocaine dose ($F(2,42) = 0.3$) on response rates (*Fig. 5 lower panel*).

Locomotor activity

1MeTIQ (25 - 50 mg/kg) did not significantly alter ($F(3,19) = 1.14$) the rats' basal locomotor activity expressed as a distance traveled during a 2-h observation period: vehicle = 1825 ± 316, 1MeTIQ (25 mg/kg) = 1517 ± 299, 1MeTIQ (50 mg/kg) = 1389 ± 249.

DISCUSSION

The main finding reported here is that exogenously applied 1MeTIQ, an endogenous substance constantly presented in the mammalian brains (18), evoked a significant decrease in the number of active-lever responding during maintenance of cocaine self-administration model and during cocaine-induced reinstatement of seeking behavior. At the same time, 1MeTIQ failed to alter the cocaine-induced cue-controlled reinstatement of drug-seeking and discriminative stimulus effects as well as food self-administration and reinstatement of food seeking.

1MeTIQ (25 - 50 mg/kg) in a dose-dependent manner decreased the cocaine self-administration (*i.e.* decreases in number of active-lever responding and cocaine infusions) maintained on the fixed ratio 5 schedule. Such an inhibitory effect can be reasoned by an increase in dopamine-related rewarding effects of cocaine since we used the training dose of cocaine on the descending limb of an inverted cocaine self-administration U-function with respect to dose (19). However, when using a fixed ratio reinforcement schedule it is difficult to draw a final conclusion since the same inhibitory responses - equivalent to increasing the training dose as rats try to compensate for the increased reinforcing effects of cocaine by making less responses - are also observed for dopamine receptor agonists (20) that enhance

dopaminergic transmission, and for direct and indirect GABA receptor agonists (21 - 23) that reduce dopaminergic transmission. Our previous *in vivo* studies have indicated that 1MeTIQ behaves as a partial agonist of dopamine receptors (*i.e.* D_2-like) as well as an antagonist at dopamine receptors (6, 12).

1MeTIQ at a dose of 50 mg/kg decreased effectively reinstatement of responding induced by a noncontingent presentation of the self-administered reinforcer (10 mg/kg of cocaine, *i.p.*). The question arises whether 1MeTIQ directly influenced the incentive motivational effects of cocaine or other mechanisms (e.g. discriminative stimulus or locomotor stimulant effects of cocaine) were involved. As shown in the present study, 1MeTIQ (50 mg/kg) neither substituted for cocaine nor significantly altered the cocaine dose-response curve in the drug discrimination model what indicates that the decreases in the expression of cocaine reinstatement evoked by 1MeTIQ cannot be linked with reduction of subjective effects of cocaine or with direct rate-decreasing effects of 1MeTIQ on operant behavior lever pressing. The latter statement is supported by the observation that the drug did not alter either the cue-induced reinstatement of cocaine-seeking or inactive lever responding. Moreover, 1MeTIQ did not significantly change basal horizontal locomotor activity in non-habituated rats (present study) or hyperactivity to acute cocaine (8; Przegaliński *et al*., submitted). Thus, it may be postulated that antagonistic effects of 1MeTIQ on the reinstatement of cocaine seeking could be attributed to its specific diminishing action on the sensitivity to the incentive motivational effects of cocaine. Further supporting the last statement, by examining the dose-range effect of 1MeTIQ - as tested in the cocaine self-administration study - on food-maintained responding we demonstrate that this drug did not evoke a general suppression of appetitive behaviors since it changed neither food self-administration nor reinstatement of food seeking.

However, it should be underlined that by producing parallel decreases in cocaine self-administration and cocaine-induced relapse, 1MeTIQ probably suppressed the motivation for drug seeking by decreasing the reinforcing effects of cocaine. To support such statement, 1MeTIQ fails to affect the discrete cues, i.e. tone + light - induced reinstatement. Since conditioned-cued reinstatement models are more analogous to relapse in humans than cocaine-primed reinstatement (24, 25), 1MeTIQ cannot be used as an effective therapeutic drug for preventing relapse. On the other hand, it seems to be a valid option for attenuating the direct rewarding properties of cocaine during the cocaine-induced "high". In the context of the potential clinical significance, 1MeTIQ has by itself no reinforcing properties since it did not produce reinstatement for cocaine seeking behavior (6) and no aversive properties as shown in the place-aversion paradigm (Antkiewicz-Michaluk *et al*., unpublished data).

1MeTIQ's inhibitory mechanism on cocaine maintained responding and relapse may include complex interaction with both dopaminergic and/or glutamatergic transmission. In fact, activation of both the dopaminergic and/or glutamatergic systems has significance in altering the maintenance of cocaine

self-administration (26 - 28) and drug-priming induced reinstatement of cocaine seeking (29 - 31). Recent findings have shown that 1MeTIQ protects the neurons against glutamate- and kainate-induced excitotoxicity (13), and such actions should be also taken into account in explanation of its anti-abuse effects.

1MeTIQ in the *in vitro* assays strongly inhibits the MAO-dependent oxidative deamination of dopamine and shifts the catabolism of dopamine towards COMT-dependent O-methylation (12) as well as directly interacts with the agonistic (active) conformation of dopamine receptors (13), while in the *in vivo* studies it antagonizes apomorphine-induced locomotor hyperactivity (12). Previous studies supported the hypothesis that MAO activity was an important factor influencing the effects of the drugs of abuse (32 - 34) found the inhibitory role of selegiline (an inhibitor of MAO) in cocaine-induced seeking behavior. Clinical studies also showed that selegiline significantly reduced craving in cocaine-dependent addicts (35).

In conclusion, our present results outline a significance of exogenously applied endogenous substance 1MeTIQ in attenuating drug-evoked relapses to cocaine as well as the direct rewarding properties of cocaine that model the cocaine-induced "high", but not cocaine subjective effects or craving. Moreover, 1-MeTIQ did not evoked a general suppression of appetitive behaviors since it changed neither food self-administration nor reinstatement of food seeking. It should be added that beyond simply reducing the rewarding effects of cocaine, 1MeTIQ produced antidepressant-like activity in rats (Frankowska and Filip, unpublished observation) what may also help in the treatment of cocaine withdrawal-evoked depression. More recently it was demonstrated that 1MeTIQ produced strong potentiation of morphine analgesia, prevented the development of morphine tolerance and inhibited expression of morphine abstinence syndrome in morphine-dependent rats (36).

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