Pharmacology Biochemistry & Behavior, Vol. 33, pp. 361–365. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Scopolamine and Methylscopolamine Differentially Affect Fixed-Consecutive-Number Performance of Male and Female Wistar Rats[1]

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Received 22 September 1988

VAN HAAREN, F., A. VAN HEST AND T. VAN HATTUM. *Scopolamine and methylscopolamine differentially affect fixed-consecutive-number performance in male and female Wistar rats.* PHARMACOL BIOCHEM BEHAV 33(2) 361–365, 1989.—Male and female Wistar rats were trained on a fixed-consecutive-number schedule in which a response on a food lever was followed by the presentation of reinforcement when at least three, but not more than seven responses had been completed on a work lever. Subjects were treated with different doses of the centrally acting cholinergic antagonist scopolamine hydrobromide or the more peripherally active cholinergic antagonist scopolamine methylbromide (0.08, 0.16 or 0.32 mg/ml/kg) once behavior had stabilized. Scopolamine hydrobromide and scopolamine methylbromide dose-dependently decreased response rates in males and females. Scopolamine methylbromide decreased response rates more than equivalent doses of scopolamine hydrobromide and the rate-suppressant effects of both drugs were more marked in males than in females. Scopolamine hydrobromide dose-dependently decreased response accuracy, but differences between males and females were not observed. Response accuracy also decreased after scopolamine methylbromide, but did not vary as a function of the dose of the drug. The decrease in response accuracy induced by both drugs was attributable to an increase in the percentage of trials with a premature switch from the work lever to the food lever. Both scopolamine hydrobromide and scopolamine methylbromide dose-dependently increased the number of premature switches. Differences between males and females were not observed. Administration of scopolamine hydrobromide and scopolamine methylbromide also decreased the number of obtained reinforcers in a dose-dependent manner. Females obtained significantly fewer reinforcers than males, while scopolamine methylbromide affected the number of obtained reinforcers to a larger extent than scopolamine hydrobromide.

Fixed-consecutive-number schedule Scopolamine hydrobromide Scopolamine methylbromide Response rates
Response accuracy Lever press Male and female Wistar rats

PHARMACOLOGICAL challenge of the central cholinergic system interferes with (short-term) memory processes. Experiments have shown that the administration of scopolamine hydrobromide (SCOP) disrupts behavior in a number of different experimental procedures such as delayed matching procedures (29,34), the radial maze (8, 24, 35) or Morris Water maze (37), Go-NoGo procedures (22) and visual and auditory discrimination procedures (9, 31, 33). It has also been shown that SCOP-treatment impairs behavior in passive avoidance procedures (6). It is thus well-established that anticholinergic treatment disrupts behavior which is predominantly controlled by external discriminative stimuli (2, 3, 28, 36).

The Fixed-Consecutive-Number (FCN) schedule has repeatedly been used to study the effects of pharmacological treatment on behavior controlled by internal discriminative stimuli. In such procedures, subjects are required to emit a specified number of responses on one (work) manipulandum in the experimental environment, before a response on another (food) manipulandum produces access to a reinforcer. The number of responses to be emitted on the work manipulandum may either be defined to

[1]The present experiment was conducted while Annemieke van Hest was supported by a grant from the Dutch Organization for the Advancement of Basic Research (NWO 560-258-024, Frans van Haaren).
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exceed a certain minimum number of responses, or it may be defined to exceed a minimum, but not to exceed a maximum number of responses (20,21). Drugs of different pharmacological classes have been shown to affect behavioral accuracy in the FCN procedure. Chlorpromazine and pimozide (neuroleptics) selectively decrease FCN accuracy, while haloperidol does not affect accuracy. d-Amphetamine and caffeine (psychomotor stimulants), as well as clonazepam and valproic acid (anticonvulsants), also decrease FCN accuracy (13, 26, 30).

The present experiment was designed to investigate whether or not pharmacological interference with the cholinergic system would disrupt behavior controlled by internal discriminative stimuli in a sex-dependent way. Male and female rats participated because of the fact that central cholinergic systems have been shown to be sexually dimorphic, (1, 15–19), and because of the fact that other experiments have shown behavioral differences between the sexes in FCN experiments (van Haaren and van Hest, submitted). Male and female Wistar rats were treated with different doses of scopolamine hydrobromide (SCOP) and scopolamine methylbromide (METHSCOP, 0.08, 0.16 and 0.32 mg/ml/kg) to investigate to what extent central anticholinergic treatment would interfere with accurate FCN performance.

METHOD

Subjects

Six female and 6 male Wistar rats had previously been trained on different fixed-consecutive-number schedules (van Haaren and van Hest, submitted). They were approximately 14 months old at the start of the present experiment. Subjects were housed in groups of three in large Macrolon cages, under a reversed light-dark cycle (lights on 7:00 p.m.–7:00 a.m.). Subjects were food-deprived on a 23-hour food deprivation schedule (11). Tap water was available at all times in the home cages.

Apparatus

Experiments took place in eight, locally constructed rat chambers (34 cm wide, 33 cm long and 37 cm high). The side walls and intelligence panel were made of black Perspex. The front door of the chamber was made of translucent Plexiglas. The floor consisted of 26 grids, spaced 1.3 cm apart. Two retractable rodent levers (2.5 cm long, 2.8 cm wide and 0.75 cm thick, when extended) were located symmetrically on each side of the pellet retrieval unit. The levers required a force in excess of 0.20 N to be operated. A Sonalert located 15 cm above each lever was then activated for 0.10 sec. A stimulus light (green on the left- and red on the righthand side of the intelligence panel) was located 9 cm directly above each lever. The pellet retrieval unit, which was centered between the two levers, could be illuminated by a white light. A houselight was mounted in the middle of the intelligence panel, 3 cm from the ceiling of the chamber. All experimental chambers were enclosed in a sound-attenuated, ventilated cabinet; the front door of this cabinet was also made of translucent Plexiglas. The chambers were connected to a PDP 11-73 microcomputer (Digital Equipment Corporation, Maynard, MA) located in an adjacent room. Experimental contingencies and data acquisition procedures were programmed using SKED-11 (27).

Procedure

Subjects had previously been trained to respond on different fixed-consecutive-number (FCN) schedules. They were thus immediately exposed to a FCN 3–7 schedule in which they were required to emit at least 3, but not more than 7 responses on the work lever, before a response on the food lever resulted in the presentation of a food pellet (45 mg BioServe). Time out (TO) was presented when subjects pressed the food lever before 3 responses on the work lever had been completed, or when subjects produced more than 7 responses on the work lever before switching to the food lever. During TO, all stimuli were extinguished and both levers were retracted from the experimental chamber for 5 sec. Sessions were terminated once 40 reinforcers had been obtained or after 30 min, whichever came first. They were conducted five days a week (Monday through Friday) during the last quarter of the subjects' dark hours.

Drug Treatment

Subjects were, first of all, intraperitoneally injected with vehicle solution (0.9% NaCl in distilled water, 1 ml/kg) during six experimental sessions. They were then injected with different doses of scopolamine hydrobromide (0.08, 0.16 or 0.32 mg/ml/kg) or scopolamine methylbromide (0.08, 0.16 or 0.32 mg/ml/kg) on Tuesdays and Fridays, 15 min before the start of the session. Drugs were freshly dissolved in vehicle solution immediately prior to testing. The volume injected was 1 ml/kg body weight. Subjects twice received all doses of each drug in randomized order within drugs. They were treated with vehicle injections on all other days of the week.

RESULTS

Response rates (responses/minute), response accuracy (the number of reinforced runs on the work lever/total number of runs), the percentage of runs not reaching the minimum response runlength on the work lever and the number of obtained reinforcers per session were, first of all, analyzed during vehicle sessions which preceded and which were interspersed between drug treatment. Males responded faster than females [males: 42.81 (SD 2.95), females: 19.57 (SD 4.62) responses/minute, $F(1,10) = 64.49$, $p<0.001$], and obtained more reinforcers during vehicle treatment [males: 40 (SD 0.0), females: 38.80 (SD 1.17), $F(1,10) = 5.96$, $p<0.035$]. Males and females were equally accurate [males: 0.73 (SD 0.11), females: 0.66 (SD 0.09), $F(1,10) = 1.34$, n.s.], while producing the same percentage of response runs which did not reach the minimum number of responses required on the work lever [males: 0.22 (SD 0.13), females: 0.32 (SD 0.09), $F(1,10) = 2.72$, n.s.]. The behavioral effects of SCOP and METHSCOP treatment were expressed as a percentage of vehicle treatment because of the behavioral differences observed during vehicle treatment. Response rates, response accuracy, the percentage of runs not reaching the minimum number of responses required on the work lever and the number of obtained reinforcers were then analyzed using analysis of variance involving the factors Sex, Drug and Dose, the latter two repeated measures within subjects (39).

Figure 1 shows response rates of male and female rats as a percentage of vehicle response rates during both SCOP (lefthand panel) and METHSCOP (righthand panel) treatment. SCOP and METHSCOP treatment dose-dependently decreased response rates for both males and females, but the effects of METHSCOP were more severe than those of SCOP [DRUG, $F(1,10) = 34.96$, $p<0.001$, DOSE, $F(2,20) = 19.91$, $p<0.001$]. The response rates of males decreased more than those of females [SEX, $F(1,10) = 8.36$, $p<0.016$].

Figure 2 shows the effects of SCOP (lefthand panel) and METHSCOP (righthand panel) treatment on response accuracy (the percentage of response runs with at least 3 but not more than 7 responses on the work lever). The analysis revealed on overall

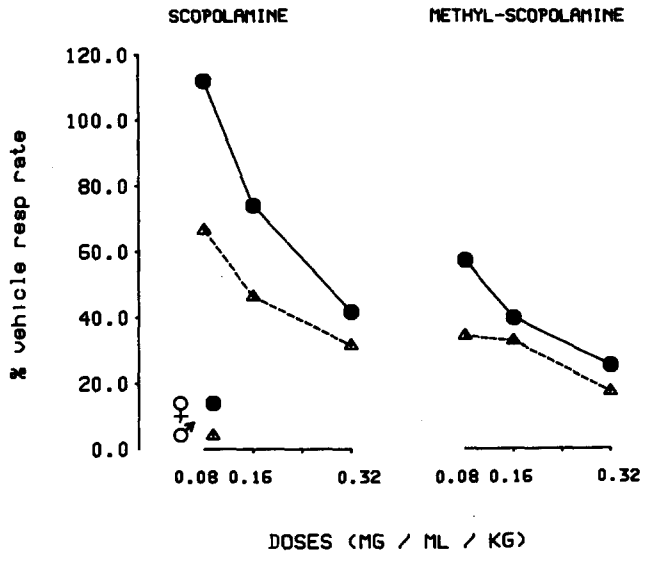

FIG. 1. Response rates (responses/minute) as a percentage of vehicle response rates for male (triangles) and female (circles) Wistar rats after scopolamine hydrobromide (lefthand panel) and scopolamine methylbromide (righthand panel) treatment.

effect of the main factor dose, indicating that response accuracy decreased dose-dependently after treatment [DOSE, $F(2,20) = 44.82$, $p<0.001$], but differences between the sexes were not observed [SEX, $F(1,10) = 0.65$, n.s.]. A significant DRUG by DOSE interaction, $F(2,20) = 9.77$, $p<0.001$, confirmed the observations in Fig. 2 that the dose effects on response accuracy were more pronounced during SCOP than during METHSCOP treatment.

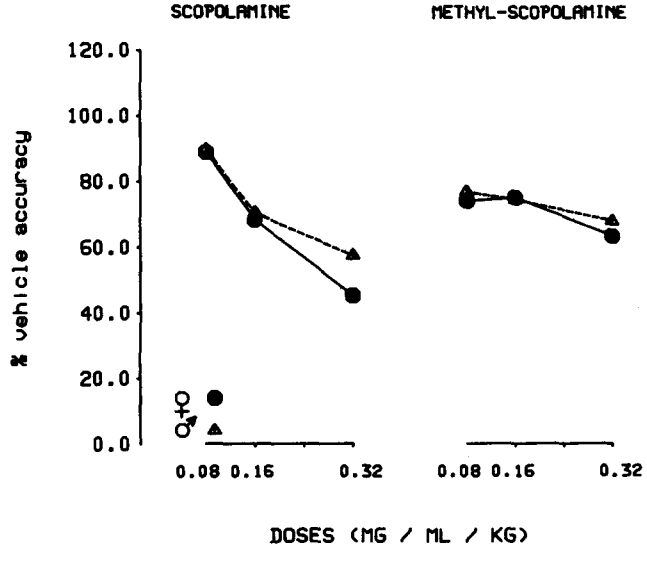

FIG. 2. Response accuracy (the number of response runs on the work lever meeting the FCN requirement/the total number of response runs on the work lever) as a percentage of response accuracy during vehicle treatment for male (triangles) and female (circles) Wistar rats after treatment with scopolamine hydrobromide (lefthand panel) and scopolamine methylbromide (righthand panel).

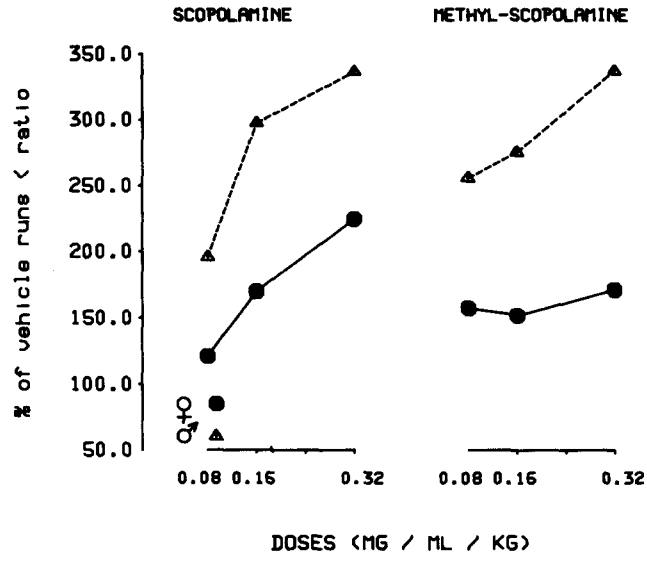

FIG. 3. The number of response runs on the work lever not reaching the minimum number of required responses as a percentage of those observed during vehicle treatment after scopolamine hydrobromide (lefthand panel) and scopolamine methylbromide (righthand panel) treatment for male (triangles) and female (circles) Wistar rats.

Figure 3 shows the effects of SCOP (lefthand panel) and METHSCOP (righthand panel) treatment on the percentage of response runs which did not reach the minimum number of responses required on the work lever. Both SCOP and METHSCOP treatment dose-dependently increased the number of response runs on the work lever which did not reach the required minimum of responses on the work lever, but a significant DRUG by DOSE interaction suggested that the DOSE effects of SCOP and METHSCOP differed [DOSE, $F(2,20) = 13.07$, $p<0.001$, and DRUG by DOSE, $F(2,20) = 4.41$, $p<0.026$]. Inspection of Fig. 3 shows that dose effects were more obvious during SCOP than during METHSCOP treatment. Sex differences were not observed, although the factor Sex almost reached significance [SEX, $F(1,10) = 3.50$, $p<0.091$].

Figure 4 shows the number of obtained reinforcers during SCOP (lefthand) and METHSCOP (righthand panel) treatment. Both SCOP and METHSCOP dose-dependently decreased the number of obtained reinforcers [DOSE, $F(2,20) = 23.13$, $p<0.001$], but the decrease was larger for females than for males [SEX, $F(1,10) = 5.76$, $p<0.037$] and more severe during METHSCOP than during SCOP treatment [DRUG, $F(1,10) = 7.75$, $p<0.019$]. A significant SEX by DOSE interaction, $F(2,20) = 4.76$, $p<0.02$, confirmed the observation in Fig. 4 that the different doses of SCOP and METHSCOP decreased the number of obtained reinforcers more for females than for males.

DISCUSSION

The present experiment has shown that treatment with SCOP and METHSCOP significantly affected the behavior of male and female Wistar rats maintained by a FCN schedule of reinforcement, showing that treatment with anticholinergic drugs not only disrupts behavior controlled by external discriminative stimuli (2, 3, 28, 37), but also behavior controlled by internal discriminative stimuli. Response accuracy decreased dose-dependently after treatment with SCOP and METHSCOP. The decrease was more

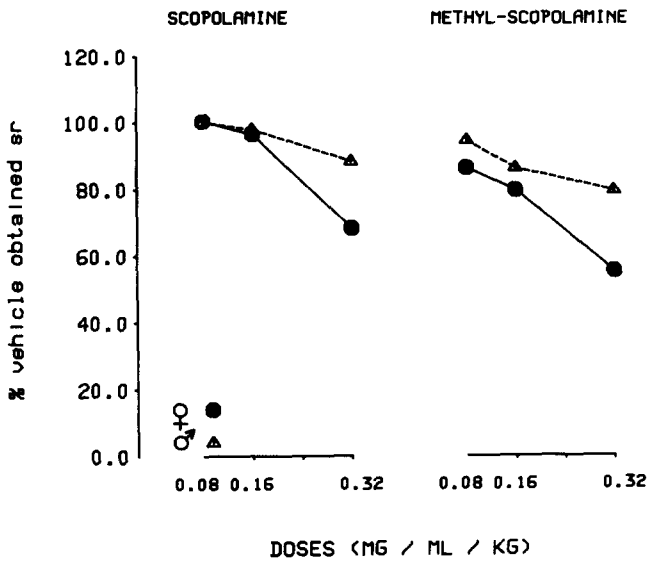

FIG. 4. The number of obtained reinforcers as a percentage of the number of reinforcers obtained during vehicle treatment after scopolamine hydrobromide (lefthand panel) and scopolamine methylbromide (righthand panel) for male (triangles) and female (circles) Wistar rats.

obvious during SCOP than during METHSCOP. The decrease in response accuracy was attributable to a dose-dependent increase in the number of response runs on the work lever which did not meet the required minimum number of responses on the work lever. SCOP and METHSCOP treatment, however, also affected response rates and the number of reinforcers obtained during each session. SCOP and METHSCOP treatment decreased response rates more for male than for female Wistar rats, while exerting the opposite influence on the number of obtained reinforcers. The results of other experiments with pigeons and monkeys (7,23) as well as those of the present experiment thus have shown that SCOP (as well as METHSCOP) treatment may significantly reduce response rates, while simultaneously increasing response variability. These results thus suggest that the effects of SCOP and METHSCOP treatment on response accuracy may have been mediated by the effects of treatment on response rates, which in turn have been caused by the peripheral effects of anticholinergic treatment, since these effects were not only observed after treatment with SCOP, but also after METHSCOP treatment. This notion is in agreement with the results of other experiments which have shown that the effects of SCOP on response accuracy are centrally mediated, whereas the effects on response rates are predominantly dependent on the peripheral actions of SCOP (5, 14, 29, 38). Other investigators previously already questioned whether or not interference with the central cholinergic system directly affects memory processes or whether the effects on memory processes are secondary to the effects on motivational or attentional processes (4). The results of the present experiment seem to confirm the hypothesis that anticholinergic treatment may interfere with memory processes through interference with other discriminative, attentional or motivational variables. Others have also suggested that such may indeed be the case, since it was shown that SCOP treatment decreased response accuracy to the same extent at all delay interval durations in a memory procedure (28). Since it may be assumed that the effects of drugs acting on memory processes increase as working load increases, it can be argued that SCOP does not directly interfere with memory processes, but that the effects of SCOP treatment on response accuracy must have been mediated by other, nonmnemonic variables.

The present experiment has also shown that anticholinergic treatment may differentially affect the behavior of male and female Wistar rats. Differential effects were not observed with respect to response accuracy. However, SCOP treatment decreased response rates more in male than in female Wistar rats, while decreasing the number of obtained reinforcers more in females than in males. The observation that response rate reduction in males exceeded the response rate reduction in females suggests that the response rate reducing effects of SCOP and METHSCOP may be a function of response rates maintained in the absence of the drug. The results of this experiment thus confirm observations by others who have also shown that SCOP treatment reduces high response rates to a larger extent than low response rates (7). The observations in the present experiment support the results from other experiments in which it was also shown that gonadal hormones may functionally affect the activity of cholinergic (van Hest, Stroet, van Haaren and Feensra, submitted), dopaminergic (31) and serotonergic systems (10). As such, these results thus suggest that it may be important to include both male as well as female subjects in experimental studies designed to investigate the behavioral effects of pharmacological manipulations.

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