Pharmacology Biochemistry & Behavior, Vol. 16, pp. 677–681, 1982. Printed in the U.S.A.

No Evidence for Cholinergic Mechanisms in the Control of Spontaneous Predatory Behavior of the Ferret

GERTRUD MEIERL AND WERNER J. SCHMIDT

Biologisches Institut der Universität Stuttgart, Ulmer Str. 227, D-7000 Stuttgart 60, F. R. G.

Received 30 March 1981

MEIERL, G. AND W. J. SCHMIDT. *No evidence for cholinergic mechanisms in the control of spontaneous predatory behavior of the ferret.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 677–681, 1982.—In a number of species there is evidence that cholinergic mechanisms participate in the control of predatory behavior. Here, the cholinomimetics arecoline (0.75 and 1.5 mg/kg), oxotremorine (12.5 and 25.0 μg/kg IM), nicotine (0.1, 0.4 and 0.8 mg/kg IM), and the cholinolytic scopolamine (0.25 and 1.0 mg/kg) were tested on the predatory behavior of the ferret. None of these compounds facilitated or inhibited capture elicitation. Oxotremorine, scopolamine and nicotine did not produce specific behavioral modifications indicating facilitation or inhibition of predatory behavior.

Predatory behavior Oxotremorine Arecoline Scopolamine Nicotine Central cholinergic mechanisms

EVIDENCE has been presented that cholinergic mechanisms are involved in the control of predatory behavior. In rats cholinomimetics and cholinolytics produce opposing effects on mouse-killing behavior (muricide). The cholinomimetic pilocarpine when systemically applied, induces muricide in non-killing rats [25] and decreases the latency of muricide in spontaneous killing rats [20]. Atropine and other cholinolytics, on the other hand, block spontaneous mouse-killing [12]. Applications of cholinomimetics to lateral hypothalamic sites may induce killing, while applications of cholinolytics block this behavior [24]. Muscarinic and nicotinic receptors may mediate opposite effects. Muricide facilitated by cholinomimetics is suppressed by antimuscarinic drugs [2,24] and spontaneous mouse-killing is blocked by nicotine [26].

A similar action of the cholinergic system on predatory behavior seems to exist for cats. In normally quiescent cats intraperitoneal injections of cholinomimetics produce biting attacks on rats [5], while pretreatment with muscarinic antagonists completely blocks the attack. Nicotine produces a dose dependent suppression of predatory behavior in cats [6]. Threshold current for electrical elicitation of predatory attack in cats is increased by the antimuscarinic drug scopolamine [15].

The conformity of these results points to homologous mechanisms for the control of predatory behavior in rats and in cats. However, recent findings contradict this view. Mouse-killing is not induced by the cholinomimetic pilocarpine in non-killing cats and not inhibited by the cholinolytic scopolamine in spontaneously killing cats [16,17]. Also antidepressants that specifically inhibit muricide as well as the predatory attack of cats elicited by hypothalamic stimulation [9], did not inhibit spontaneous predatory attack of cats [17] and of ferrets [22]. Some doubt has been thrown even upon the specificity of the muricide inhibiting action of atropine sulfate. This cholinergic antagonist seems to suppress muricide only at high doses which produce a general suppression of behavior [1]. Thus, more detailed investigations of cholinergic influences on the predatory behavior are necessary. Here, the effects of the cholinomimetics arecoline, oxotremorine, nicotine, and of the cholinolytic scopolamine on the predatory behavior of the ferret are examined.

METHOD

Predators were seven adult male ferrets (*Putorius furo* L.), kept in natural day-night cycle with free access to food and water. As live prey, 220 g Wistar rats were used. The experiments were carried out in a circular arena (dia., 2 m) familiar to the animals. The ferrets were introduced two minutes after the prey. The predatory behavior was recorded with a video system. From the recordings time courses of 7 regularly occurring behavioral parameters were assessed and transferred to a computer: (1) The latency from introduction of the ferret (start of the experiment) to the perception of the prey. Perception of the prey is entered in the record when the ferret, after initial sniffing, oriented the head towards the prey and remined motionless for a short time (1–2 sec). (2) The latency from perception to the death of the prey. (3) The latency from perception to the first bite. (4) From the first bite to the death of the prey. (5) Number and duration of bites necessary to adjust the placement and to destroy the basal skull or spinal cord. (6) The number of escapes of the prey, and (7) The number of ferrets that attacked and killed the prey within 5 minutes. After the death of the rat the ferret left the prey without eating it (see also [21]).

To account for season dependent variations of the predatory behavior, pre- and postdrug control trials were carried

out 24 hours before and after drug application (if not otherwise specified in the results). For statistical evaluation the Wilcoxon matched pairs signed-rank test (two-tailed) was used. The test values were compared to the pre- and postdrug control values and considered as significantly different when $p \leqq 0.05$ in both comparisons.

All drugs were dissolved in 0.9% NaCl and applied IM in approximately 1 ml volumes. Doses are expressed in terms of the base.

Arecoline (0.75 and 1.5 mg/kg) was injected 3 minutes and oxotremorine (12.5 and 25.0 μg/kg) 20 minutes pretest. To minimize their peripheral effects atropine methyl nitrate (0.25 and 0.5 mg/kg) was applied 5 minutes before the cholinomimetics. Scopolamine (0.25 and 1.0 mg/kg) was applied 1 and 3 hours before the tests, and nicotine (0.1, 0.4 and 0.8 mg/kg) was injected 5 to 10 minutes pretest.

TABLE 1

FERRETS KILLING THE PREY
(TRIALS WITH CAPTURE/TRIALS CONDUCTED)

		Predrug controls	Drug	Postdrug controls
Oxotremorine	12.5 μg/kg	13/14	7/7	13/14
Oxotremorine	25.0 μg/kg	14/14	6/7	13/14
Arecoline	0.75 mg/kg	0/3	0/3	0/3
Arecoline	1.50 mg/kg	5/22	2/11	4/18
Scopolamine	0.25 mg/kg	19/20	9/10	14/14
Scopolamine	1.00 mg/kg	11/12	5/6	5/6
Nicotine	0.1 mg/kg	13/14	5/7	
Nicotine	0.4 mg/kg		5/7	
Nicotine	0.8 mg/kg		5/7	7/14

RESULTS

Oxotremorine

Pilot experiments indicated that the cholinomimetic oxotremorine produces peripheral parasympathetic effects, e.g., salivation and defecation. In addition, the ferrets scratched themselves vigorously and dragged their hindlegs. Under both doses an increased reactivity to noises and to optic signals was conspicuous. To minimize the peripheral effects, all ferrets were pretreated five minutes before oxotremorine injections with atropine methyl nitrate (0.25 and 0.50 mg/kg IM), a quaternary muscarinic antagonist that does not readily cross the blood brain barrier [14]. While salivation and defecation disappeared with this pretreatment, scratching and temporary dragging of the hindlegs still occurred, but less defined. These drug effects seemed to depend on the motivational state of the animals, because they did not appear whenever the ferrets performed an active behavior like sniffing, digging or predation. The increased reactivity to optic and acoustic signals, however, was not changed by the treatment with atropine methyl nitrate and was still observed during predation.

Both doses of oxotremorine (12.5 and 25.0 μg/kg) caused no profound changes in the percentage of rats that were killed (Table 1). In a dose of 25.0 μg/kg it did not influence any of the evaluated parameters of predatory behavior. Under 12.5 μg/kg, latency from perception to the death of the prey was significantly shorter (Table 2). Since the latency of attack (time from perception to the first bite) was not shortened this change seemed to be due to an increased biting frequency which reduced the duration from the first bite to the death of the prey. The mean number of bites, necessary to kill the prey, was not changed significantly by oxotremorine.

Arecoline

The peripheral effects of arecoline were similar to oxotremorine and minimized by pretreatment with atropine methyl nitrate (0.25 and 0.5 mg/kg). The proportion of ferrets that killed the prey did not increase (Table 1). The behavioral parameters could not be statistically evaluated since the number of trials with capture was too small.

Scopolamine

The antimuscarinic compound scopolamine produced parasympatholytic effects such as dilation of the pupils, decreased salivation and in higher dosages, visual disorders due to deficient accommodation. Under both doses (0.25 and 1.0 mg/kg) the pupils of the ferrets were opened widely and their mouths were dry. Scopolamine postdrug control trials were not conducted until pupil dilation was totally reduced (120 hr postdrug). Capture elicitation was not changed under scopolamine, and although 1.0 mg/kg produced marked motor impairment in addition to the other side effects, rats were killed in 5 of the 6 tests (Table 1). Under both doses perception of the prey was delayed and the rats were able to escape more often (Table 2). All other analyzed parameters were not changed under 0.25 mg/kg. But 1.0 mg/kg scopolamine increased the time from perception to the first bite on average (predrug: 11.6 sec, drug: 44.2 sec, postdrug: 16.9 sec) and consequently the time from perception to the death of the prey. The prolonged killing latency was not surprising, as this dose already produced motor debilitation which caused the ferrets often to lie down.

Nicotine

While peripheral effects did not appear at all under 0.1 mg/kg nicotine, injections of 0.4 and 0.8 mg/kg increased salivation in nearly all ferrets. With one exception, no signs of nausea have been observed. Since nicotine is reduced by the organism to the half of the original dose within 2 hours [10], the nicotine tests were separated by 24 hr with no additional interim control trials. Predrug control trials before 0.1 mg/kg and postdrug control trials after 0.8 mg/kg served as controls for all three doses.

Five of the 7 ferrets killed the prey under the 3 doses of nicotine (Table 1). Although two animals were debilitated under 0.8 mg/kg nicotine, one of these two killed its prey. Nicotine caused no specific modification of other behavioral parameters analyzed.

The experiments with nicotine were conducted on 7 successive days in April. During this time a seasonal shift of killing frequency occurs. The percentage of ferrets that attacked and killed the prey decreased from 100% to 42.9%. The low baseline of capture elicitation persisted during summer and returned to its prenicotine baseline in autumn and winter. During the three days of tests with nicotine the percentage of ferrets that killed the prey remained constant

TABLE 2

EFFECTS OF SCOPOLAMINE (0.25 mg/kg) AND OXOTREMORINE (12.5 μg/kg) ON SOME PARAMETERS OF PREDATORY BEHAVIOR

	Scopolamine			Oxotremorine		
	Predrug controls	Tests	Postdrug controls	Predrug controls	Tests	Postdrug controls
Latency						
from start to perception of the prey/sec	2.3	5.3†	2.1	3.4	4.7	4.6
from perception to death of the prey/sec	55.0	79.6	92.3	73.9	39.6*	111.2
from perception to the first bite/sec	18.6	19.6	11.3	12.9	12.4	16.8
from the first bite to death of the prey/sec	36.2	61.9	81.0	62.1	45.0*	95.8
Bites per second	0.25	0.20	0.26	0.39	0.65*	0.40
Escape of the prey (mean)	1.3	4.3*	2.4	1.9	1.3	3.2
Number of trials	19	9	14	13	7	13

*Significantly different from both controls, $p<0.05$.
†In comparison with predrug controls $0.05<p<0.01$, and with postdrug controls $p=0.05$.

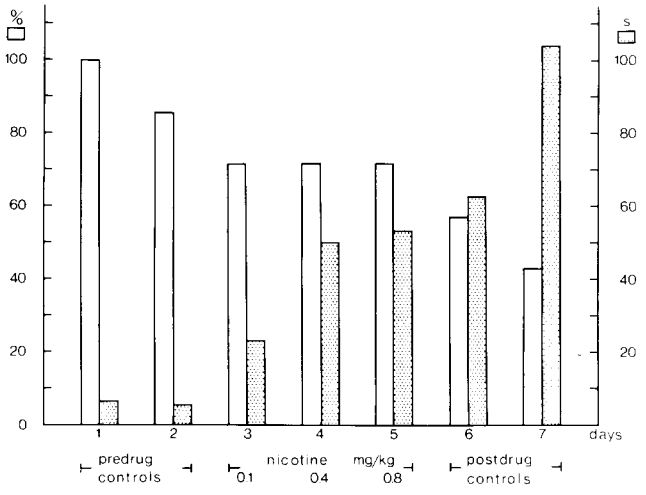

FIG. 1. Blank columns: Percentage of ferrets that killed the prey. Dotted columns: Cumulative duration of averting from the prey.

(Fig. 1). Thus, if there existed any effect of nicotine, it was rather a facilitatory than an inhibitory one.

With decreasing numbers of ferrets that killed, the cumulative duration of averting from the prey increased. The mean of duration of averting was only 6 seconds at the time when all ferrets killed, and it increased during this week to 104 seconds (Fig. 1).

DISCUSSION

The nicotinic or antimuscarinic compounds used in this study did not inhibit elicitation of the predatory behavior of the ferret and the cholinomimetics did not facilitate it. This contrasts with previous findings [6,15] which showed an inhibition of the predatory attack of the cat using similar doses of scopolamine and nicotine, and to the finding that the cholinomimetics arecoline and oxotremorine stimulate biting attack on rats in the cat [5]. The failure of arecoline and oxotremorine to stimulate capture elicitation in the ferret does not seem to be due to vegetative disorders, since the peripheral effects were minimized by the quaternary muscarinic antagonist atropine methyl nitrate which was injected before the cholinomimetics. Although scratching and temporary dragging of the hindlegs still occurred after this pretreatment, the behavioral patterns, the animals were motivated for, were not hindered, i.e., scratching and leg-dragging never occurred during predation.

The modifications of behavioral parameters observed under anticholinergic and cholinergic treatments, yield no evidence for a specific influence on the control of predatory behavior. The increased killing latency under 1.0 mg/kg scopolamine was due to motor impairment which made the ferrets often lie down. After reduction of the dosage to 0.25 mg/kg no debilitating motor effects were observed and killing latency did not differ from control values. However, two behavioral changes occurred under both doses and therefore seemed to be scopolamine actions: rats did escape more often and the latency until perception of the prey was prolonged. Under 0.25 mg/kg scopolamine rats were able to escape 4 to 5 times, whereas during control trials the mean number of escapes was between 1 and 3. This was not due to imprecise biting since mainly bites were observed that pointed to the rat's neck. In addition, the number of bites necessary to kill the prey was not increased, bites may have been weaker.

Oxotremorine, in a dose of 25 μg/kg did not show any effects. At 12.5 μg/kg it raised the biting frequency. Consequently two other parameters, latency from perception to death of the prey and from the first bite to the death of the

prey decreased. Though a facilitation of biting is produced by oxotremorine, in our opinion the degree of specificity of this drug effect does not justify to assume a cholinergic link to the control of predatory behavior of the ferret. Scopolamine and oxotremorine do rather seem to act indirectly on predatory behavior by altering the state of "wakefulness" or "excitation" [7,11]. This presumption may be confirmed by the observation that the animals showed an increased reactivity to all acoustic and optic signals under oxotremorine.

Nicotine did not modify any of the behavioral parameters. The continuous decrease of killing frequency during this series of experiments reflects the seasonal changes of predatory behavior (Fig. 1). Such variations have been observed for several years [21]. Male ferrets do kill nearly 100% of the prey in winter whereas in summer predation is elicited in only 50% of the experiments.

In summary our present data do not show facilitation of predatory behavior by cholinergic compounds or inhibition by anticholinergic compounds or nicotine. Moreover, the observed modifications of behavioral parameters do not seem to be due to cholinergic influences on central mechanisms which are specifically involved in the control of the predatory behavior of the ferret.

The obvious differences in the results obtained from the experiments with cats [5, 6, 15] and those described here with ferrets, raise the question if the described behaviors have homologous mechanisms. Berntson [6] reported that the biting attack of the cat, induced by arecoline, is always accompanied by restlessness, agitation and threat behavior as hissing and growling. Nicotine blocked not only biting attack but also threat behavior. As already mentioned [6] further work is necessary to determine the nature of muscarinic stimulation and nicotinic blockade of predatory attack in the cat. Other studies have shown that intracranial injections of cholinomimetics induce emotional-affective responses [19] and make cats attack rats without killing them [4]. Since the biting attack of the cat (described in [5] and [6]) comprises components of affective aggression [8,18], it may be of other nature than the predatory behavior of the ferret described here. This may be one reason for the differing pharmacological results obtained with cats and ferrets.

Other experiments with cats which point to a cholinergic link in the central control of predatory behavior have examined the action of cholinomimetics and cholinolytics on electrically stimulated attack behavior. However, electrically induced attack behavior may have other pharmacological properties than spontaneous predatory behavior. Scopolamine, for instance, does not inhibit spontaneous predatory behavior in cats [17] but inhibits electrically elicited predatory attack in cats [15]. Amphetamine inhibits spontaneous predation in rats [3,13], cats [17] and ferrets [21], but facilitates electrically elicited predatory attacks in cats [23]. The contradictory results may therefore be due to different procedures used to obtain predatory attack.

Our results on the predatory behavior of the ferret resemble the findings of Leaf [16,17] who reported that in contrast to the rat, spontaneous predatory behavior of cats is not inhibited by scopolamine [17] and not stimulated by pilocarpine [16]. In addition, also antidepressants, known to specifically inhibit muricide [13], do not inhibit the spontaneous predatory behavior of cats [17] and ferrets [22]. These findings may suggest related mechanisms in the cat and the ferret but different ones in the rat.

In conclusion, the data presented on the predatory behavior of the ferret do not favor the assumption of a cholinergic link in the brain systems that control spontaneous predatory behavior of the ferret.

ACKNOWLEDGEMENT

With financial support by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Albert, D. J. Suppression of mouse killing by lateral hypothalamic infusion of atropine sulfate in the rat: A general behavioral suppresssion. *Pharmac. Biochem. Behav.* **12:** 681–684, 1980.
2. Bandler, R. J. Cholinergic synapses in the lateral hypothalamus for the control of predatory aggression in the rat. *Brain Res.* **20:** 409–424, 1970.
3. Barr, G. A., J. L. Gibbons and W. H. Bridger. Inhibition of rat predatory aggression by acute and chronic D- and L-amphetamine. *Brain Res.* **124:** 565–570, 1977.
4. Beleslin, D. B. and R. Samardzic. Muscarine- and carbachol-induced aggressions: Fear and irritable kinds of aggressions. *Psychopharmacology* **55:** 233–236, 1977.
5. Berntson, G. G. and S. F. Leibowitz. Biting attack in cats: Evidence for central muscarinic mediation. *Brain Res.* **51:** 366–370, 1973.
6. Berntson, G. G., M. S. Beattie and J. M. Walker. Effects of nicotinic and muscarinic compounds on biting attack in the cat. *Pharmac. Biochem. Behav.* **5:** 235–239, 1976.
7. Buser, P., L. Cook, C. Giurgea, E. Jacobsen, O. S. Ray, M. Richelle, A. P. Silverman, L. Stein and Z. Votava. Animal behavior. In: *The Neuroleptics. Modern Problems in Pharmacopsychiatry.* Basel: Karger, 1970, pp. 85–108.
8. Delini-Stula, A. and A. Vassout. Differential effects of psychoactive drugs in aggressive responses in mice and rats. In: *Psychopharmacology of Aggression,* edited by Merton Sandler. New York: Raven Press, 1979, pp. 41–60.
9. Dubinsky, B., J. K. Karpowicz and M. E. Goldberg. Effects of tricyclic antidepressants on attack elicited by hypothalamic stimulation: Relation to brain biogenic amines. *J. Pharmac. exp. Ther.* **187:** 550–557, 1973.
10. Forth, W., D. Henschler and W. Rummel. *Allgemeine und spezielle Pharmakologie und Toxikologie.* Zürich: Bibliographisches Institut, 1980, p. 633.
11. Grossman, S. P. Behavioral and electroencephalographic effects of microinjections of neurohumors into the midbrain reticular formation. *Physiol. Behav.* **3:** 777–786, 1968.
12. Hoffmeister, F., H. Kreiskott and W. Wirth. Untersuchungen mit zentral wirksamen Anticholinergika. Arzneimittelforschung **14:** 482–486, 1964.
13. Horovitz, Z. P., J. J. Piala, J. P. High, J. C. Burke and R. C. Leaf. Effects of drugs in the mouse-killing (muricide) test and its relationship of amygdaloid function. *Int. J. Neuropharmac.* **5:** 405–411, 1966.
14. Innes, I. R. and M. Nickerson. Atropine, scopolamine, and related antimuscarinic drugs. In: *The Pharmacological Basis of Therapeutics,* edited by L. S. Goodman and A. Gilman. New York: Macmillan, 1975, p. 516.
15. Katz, R. J. and E. Thomas. Effect of scopolamine and α-methylparatyrosine upon predatory attack in cats. *Psychopharmacology* **42:** 153–157, 1975.
16. Leaf, R. C. and D. J. Wnek. Pilocarpine, food deprivation, and induction of mouse killing by cats. *Pharmac. Biochem. Behav.* **9:** 439–444, 1978.

17. Leaf, R. C., D. J. Wnek, S. Lamon and P. E. Gay. Despite various drugs, cats continue to kill mice. *Pharmac. Biochem. Behav.* **9:** 445–452, 1978.
18. Leyhausen, P. Das Abwehrverhalten. In: *Katzen, eine Verhaltenskunde*, edited by P. Leyhausen. Berlin: Parey, 1979, p. 69.
19. MacLean, P. D. and J. M. R. Delgado. Electrical and chemical stimulation of frontotemporal portion of limbic system in the waking animal. *Electroenceph. clin. Neurophysiol.* **5:** 91–100, 1953.
20. McCarthy, D. Mouse-killing induced in rats treated with pilocarpine. *Fedn Proc.* **25:** 385, 1966.
21. Schmidt, W. J. Effects of d-amphetamine, maprotiline, L-dopa, and haloperidol on the components of the predatory behavior of the ferret, putorius furo L. *Psychopharmacology* **64:** 355–359, 1979.
22. Schmidt, W. J. and G. Meierl. Antidepressants and the control of predatory behavior. *Physiol. Behav.* **25:** 17–19, 1980.
23. Sheard, M. H. The effects of amphetamine on attack behavior in the cat. *Brain Res.* **5:** 330–338, 1967.
24. Smith, D. E., M. B. King and B. G. Hoebel. Lateral hypothalamic control of killing: Evidence for a cholinoceptive mechanism. *Science* 167: 900–901, 1970.
25. Vogel, J. R. and R. C. Leaf. Initiation of mouse killing in nonkiller rats by repeated pilocarpine treatment. *Physiol. Behav.* **8:** 421–424, 1972.
26. Waldbillig, R. J. Suppressive effects of intraperitoneal and intraventricular injections of nicotine on muricide and shock-induced attack on conspecifics. *Pharmac. Biochem. Behav.* **12:** 619–623, 1980.