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Effects of Selective Central Muscarinic Blockade on Schedule-Controlled Behavior and on the Rate-Decreasing Effects of Physostigmine[1]

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HYMOWITZ, N. AND H. E. BREZENOFF. *Effects of selective central muscarinic blockade on schedule-controlled behavior and on the rate-decreasing effects of physostigmine.* PHARMACOL BIOCHEM BEHAV **21**(1) 109–115, 1984.—N-(4-diethylamino-2-butynyl)-succinimide, or DKJ-21, is a muscarinic receptor antagonist with a high degree of selectivity for the central nervous system. In the present study of 6 rats maintained under a fixed-interval 50-sec schedule of food reinforcement, atropine and methylatropine reduced responding in a dose dependent manner, while DKJ-21 had little or no effect. Our findings suggest that the suppression caused by atropine and methylatropine may be the result of the dry mouth induced by these agents. Doses of DKJ-21 which had no effect on schedule performance antagonized the rate-lowering effects of physostigmine in all of the animals. Neither atropine nor methylatropine consistently antagonized the inhibitory effects of physostigmine. Some antagonism may be inferred, however, from the findings that response rates were suppressed less by combinations of atropine and physostigmine than by either drug alone.

DKJ-21 Atropine Methylatropine Physostigmine Schedule-controlled behavior Rats

CENTRALLY acting muscarinic antagonists such as atropine generally decrease rates of schedule-controlled responding in pigeons [12, 17, 18], monkeys [3], mice [19] and rats [1], although atropine occasionally has been reported to increase responding during the early portion of the interval with fixed-interval schedules [1,19]. In spite of its inhibitory effects, atropine has been reported to antagonize the suppressive effects of physostigmine [3, 17, 18] and oxotremorine on responding [12]. In contrast, methylatropine, which does not readily enter the central nervous system (CNS), is less effective in suppressing response rates [12,17] and antagonizing the effects of physostigmine [17,18]. It is thereby assumed that the behavioral and antagonistic effects of atropine are due to central influences [18].

N-(4-diethylamino-2-butynyl)- succinimide (DKJ-21) is a muscarinic antagonist with a high degree of central selectivity [5,6]. It can abolish the central tremorigenic effect of oxotremorine, a direct acting muscarinic agonist, at doses which have no mydriatic activity [5,6]. Little work has been done with this compound in behavioral studies. Slater [16] reported that intracerebroventricular injections of DKJ-21 significantly reduced brain acetylcholine levels but had no effect on maze performance in rats. The present study extends the analysis of DKJ-21 to effects on schedule-controlled behavior and antagonism of the behavioral effects produced by physostigmine. The responses produced by DKJ-21 were compared to those produced by atropine, which has both central and peripheral actions, and methylatropine, which is restricted to the periphery.

METHOD

Subjects

Six experimentally naive male Sprague-Dawley rats initially weighing 325–375 g were maintained at 80% of free-feeding weight. They were housed individually with water freely available in the home cage unless specified otherwise.

Apparatus

A Grason-Stadler sound attenuating operant rat chamber, Model 1101, contained a response lever, pellet dispenser, white masking noise, and fan. Programming was accomplished by electromechanical equipment and responses were monitored by counters and a cumulative recorder. Two jewelled lights, located above and to the left and right of the response lever, served as the conditioned stimulus (CS). Noyes food pellets (0.045 g) served as reinforcers.

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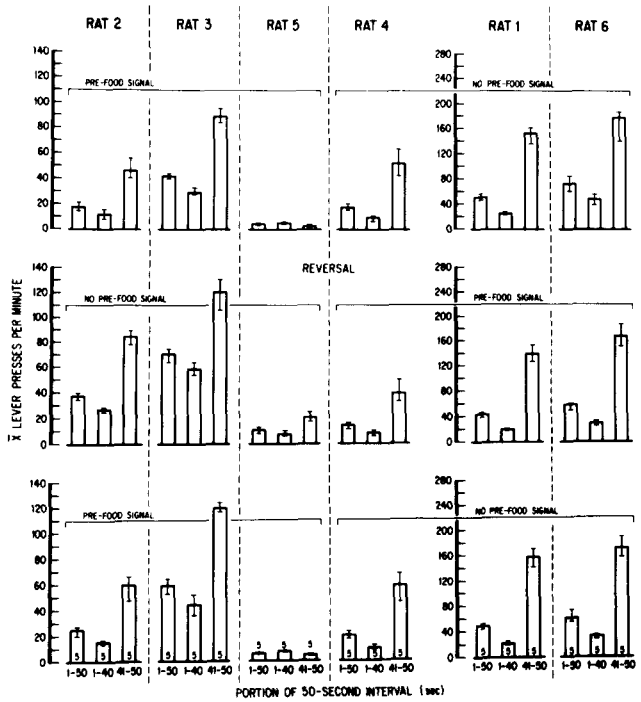

FIG. 1. Control mean lever presses per minute during the entire 50-second interval (1–50), the first 40 seconds (1–40), and the last 10 seconds (41–50), of the interval. Each bar represents the mean±SEM of the last five days during either baseline (upper section), reversal (middle section), or return to baseline (lower section). The pre-food signal (CS) was presented during seconds 41–50.

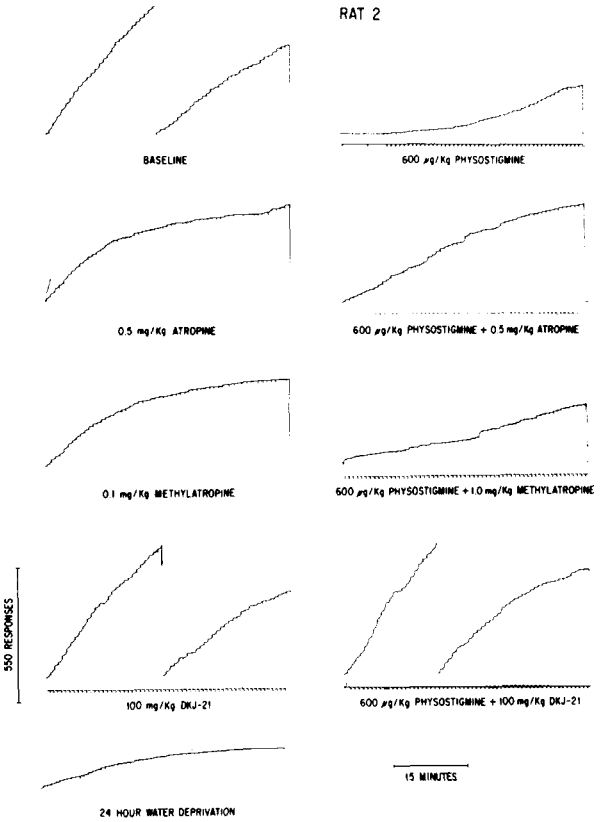

FIG. 2. Representative cumulative records of responding for Rat 2 during selected experimental conditions. Vertical movement of the response pen indicates lever presses; the downward pip indicates pellets. The deflection of the event pen indicates the final 10 sec of the 50-sec interval. The experimental conditions shown are, top left to bottom: 0.5 mg/kg atropine, 0.1 mg/kg methylatropine, 100 mg/kg DKJ-21, and 24-hr water deprivation. From top right to bottom are: 0.6 mg/kg physostigmine, 0.6 mg/kg physostigmine plus 0.5 mg/kg atropine, 0.6 mg/kg physostigmine plus 1.0 mg/kg methylatropine, and 0.6 mg/kg physostigmine plus 100 mg/kg DKJ-21.

Drugs

The following drugs were employed: atropine sulfate, atropine methylbromide, DKJ-21·HCl and physostigmine salycilate. Drugs were dissolved in saline and injected IP in a volume of 0.1 ml/kg. All doses refer to the salt.

Procedure

Phase 1. Initial acquisition and baseline. Following reduction to 80% of free-feeding weight and lever-press shaping, the behavior of the animal was studied for 25–30 50-min sessions under a FI 50-sec schedule of food reinforcement. For rats 2, 3 and 5, a 10-sec CS (onset of two jewelled lights in otherwise dark chamber) was presented during the last 10 sec of the 50-sec interval. Offset of the CS was simultaneous with food availability so that the next response produced a food pellet. (The latter schedule might alternatively be described as a multiple time-out 40-sec, time-out 10-sec, fixed-ratio-1 schedule.) For rats 1, 4, and 6, the CS was not presented and food availability was unsignaled. The start of the daily sessions was indicated by the onset of white masking noise.

Phase 2. Effects of atropine, methylatropine, and DKJ-21 on FI-controlled response rates. Following acquisition of stable rates of lever-pressing, responses were studied under a range of doses of atropine, methylatropine, and DKJ-21. The drugs were administered IP 15 min prior to the start of the experimental sessions. At least two drug-free days preceded each drug session; drugs were not administered until response rates recovered to pre-drug levels. The animals were exposed first to an ascending and then to a descending series of atropine doses (0.05–8.0 mg/kg). Next, the rats were studied under ascending series of methylatropine (0.05–1.0 mg/kg) and DKJ-21 (10–140 mg/kg). Behavioral effects of these compounds were redetermined in Phase 3.

Phase 3. Antagonism of the rate-decreasing effects of physostigmine. After 5–10 drug-free sessions, response rates were studied under an ascending series of doses of physostigmine salicylate administered IP 5 min prior to the session. The dose was increased until response rates were consistently suppressed at the same dose on 2–4 successive occasions. Tolerance to the suppressive effects of physostigmine was observed on several occasions, and it was necessary to increase the dose and/or the interval between doses.

The order of assessing antagonism was DKJ-21, atropine and methylatropine. The drug was administered 15 min prior to the session (i.e., 10 min prior to physostigmine). The effects of physostigmine alone and in combination with either DKJ-21, atropine, and methylatropine were assessed according to an ABA design to make sure that tolerance to physostigmine did not develop. That is, physostigmine was ad-

TABLE 1

EFFECTS OF ATROPINE, METHYLATROPINE AND DKJ-21 ON RESPONSE RATE FOR RATS EXPOSED TO SIGNALED AND UNSIGNALED FOOD REINFORCEMENT

	Signaled†		Unsignaled	
Dose*	1–40 sec	41–50 sec	1–40 sec	41–50 sec
		Atropine		
0.05	117‡	102	85	94
0.1	89	87	93	100
0.5	62	72	66	63
1.0	47	38	50	57
2.0	45	31	52	43
4.0	39	29	21	16
		Methylatropine		
0.05	78	77	53	58
0.1	37	33	55	61
0.5	33	25	20	35
		DKJ-21		
5.0	105	97	108	99
20.0	95	109	89	90
60.0	89	99	90	113
120.0	77	109	102	89

*Doses are given in mg/kg.
†Three rats each in the signaled and unsignaled groups.
‡Each value is the mean of 2–4 experiments in each of the 3 rats expressed as percent of the preceding baseline session.

ministered alone prior to and following administration in combination with the antagonist. A minimum of two drug-free days intervened between drug sessions.

Phase 4: Effects of water deprivation on response rates. To study the influence of xerostomia (dry mouth) on response rates, the home cage water bottle was removed for 24 hours prior to an experimental session (2–4 times/rat). The bottle was returned following the session, and a minimum of two sessions with access to water in the home cage separated water deprivation sessions.

Phase 5: Effects of CS reversal on response rates. Rats normally exposed to signaled food availability were studied for 10 successive sessions without the CS, while rats normally studied under unsignaled food availability were studied for 10 sessions with the CS (Reversal). An additional block of 10 sessions followed in which the original signal conditions were reinstated.

RESULTS

Phase 1. Initial Acquisition and Baseline

Control response rates are shown in Fig. 1, and representative cumulative records for one animal (Rat 2) are presented in Fig. 2. For most of the animals, response rates increased somewhat over the course of the study. All animals except Rat 5 revealed a characteristic FI pattern of responding, with low rates in the initial portion of the interval and higher rates towards the end (Fig. 1). This pattern was maintained whether or not the CS was present. Rat 5 maintained a low rate of responding throughout the interval, often not emitting a response until the CS terminated and food became available. When the CS was removed for Rats 2, 3 and 5 (Reversal), response rates in each portion of the interval increased; when the CS was presented for the first time to Rats 1, 4 and 6, response rate decreased slightly in each portion of the interval (Fig. 1). These reversal experiments show that the CS exerted a positive influence on behavior.

Phase 2. Effects of Atropine, Methylatropine, and DKJ-21 on FI-Controlled Responding

The effects of the drugs on behavior were virtually the same whether or not food availability was signaled. Therefore, data for all the animals will be considered together. Figure 3 and Table 1 show effects of drugs as a percent of the previous predrug session response rate. Atropine and methylatropine decreased responding in a dose-dependent manner whether or not food was signaled. As the dose of atropine increased, response rates decreased in each portion of the interval. At most doses, rate-dependent effects were not observed; response rates in each portion of the interval were decreased similarly. At 0.05 mg/kg atropine, however, some animals revealed an increase in responding early in the interval, while response rates later in the interval were unaffected. In contrast to these effects, DKJ-21 had little or no effect on response rates, even when given in doses of 120 mg/kg.

Figure 2 shows representative cumulative records of responses for Rat 2 following 0.5 mg/kg atropine, 0.1 mg/kg

TABLE 2
EFFECT OF 24-HOUR WATER DEPRIVATION ON RESPONSE RATES

Rat	N*	Baseline 1–50†	Baseline 1–40	Baseline 41–50	Water Deprivation 1–50	Water Deprivation 1–40	Water Deprivation 41–50
			Signaled				
2	2	24.2‡	12.6	70.6	11.0	7.9	49.8
3	3	65.4	49.3	129.8	44.4	29.8	102.6
5	3	5.1	5.4	3.8	4.1	4.6	1.9
Percent of baseline					62.9	62.9	75.5
			Unsignaled				
1	2	49.1	21.9	157.9	33.1	16.7	98.6
4	3	17.8	9.8	49.7	10.9	6.7	28.1
6	4	68.2	39.8	181.9	45.9	22.0	141.8
Percent of baseline					66.6	63.3	68.9

*Number of experiments per rat.
†Portion of the interval.
‡Values are expressed as responses/min.

methylatropine, and 100 mg/kg DKJ-21. Atropine and methylatropine decreased response rates throughout the 50-sec interval, with no obvious increase in response rates early in the interval. As the session progressed, response rates declined further, and the time between the end of the CS and the response that produced the next pellet of food increased. Response rates were maintained at control levels under DKJ-21.

Unchewed and partly chewed food pellets were found in the excreta tray. Since both atropine and methylatropine produce xerostomia, we speculated that dry mouth may have caused difficulty in swallowing the food. This in turn could result in a decrease in "motivation" for lever-pressing. To test this hypothesis we examined the effects on responding produced by 24-hour water deprivation. As shown in Table 2, 24-hour water deprivation also reduced response rates, with relatively more reduction in the early portion of the interval. Numerous food pellets were found in the excreta tray and, as shown in Fig. 2 (lower left panel), response rates decreased as the session progressed. The time between CS termination and a response also increased. These findings support the hypothesis that the lowered response rates following atropine and methylatropine may have been due, at least in part, to xerostomia.

Phase 3. Antagonism of the Rate-Decreasing Effects of Physostigmine

Presession administration of physostigmine reduced response rates in each portion of the 50-sec interval whether or not food was signaled (Fig. 4). Threshold effects were observed at doses of 50–100 μg/kg. Considerable variability was noted and tolerance generally developed, especially at the lower doses. The dose and/or the interval between doses was increased until stable effects were obtained.

To test antagonism, doses of physostigmine were selected which consistently produced submaximal suppression of responding. These doses ranged between 250 and 600 μg/kg in the different subjects. The degree of suppression produced by physostigmine did not decrease in the presence of atropine or methylatropine (Fig. 3). Some antagonism between atropine and physostigmine may be inferred from the fact that response rates were suppressed to the same extent or somewhat less by the combination of physostigmine and atropine than by atropine administered alone (Fig. 4). In addition, fewer food pellets were found in the excreta tray.

Rat 2 revealed partial blocking of the suppressive effects of physostigmine at several doses of atropine. As shown in Fig. 2 (second panel on right), the combination of atropine and physostigmone yielded a moderate rate of responding which was maintained throughout the session. Note that the scalloped response rate was restored as responses in each portion of the interval increased in frequency. Fewer food pellets were found beneath the grid, suggesting that xerostomia was not as severe as under atropine alone.

Methylatropine had no obvious blocking effects (Fig. 1). In some rats, the combination of methylatropine and physostigmine reduced response rates more than either compound alone.

In contrast to atropine and methylatropine, DKJ-21 clearly antagonized the rate-reducing effects of physostigmine in each animal (Fig. 4). In each case, antagonism of physostigmine was achieved with doses of DKJ-21 which had little or no effect on response rates when administered alone. As shown in Fig. 1 (bottom right panel), 100 mg/kg DKJ-21 restored the "scalloped" response rate of Rat 2 to predrug levels. In the other animals the effective dose ranged between 40 and 80 mg/kg.

DISCUSSION

Cholinesterase inhibitors disrupt learned behavior in a variety of experimental paradigms [17,18]. In the present study, physostigmine suppressed operant responding maintained by a Fixed-Interval 50-sec schedule of food reinforcement. Both atropine and methylatropine also suppressed the rate of responding. Methylatropine does not readily enter the brain, suggesting that the reduction is due to a

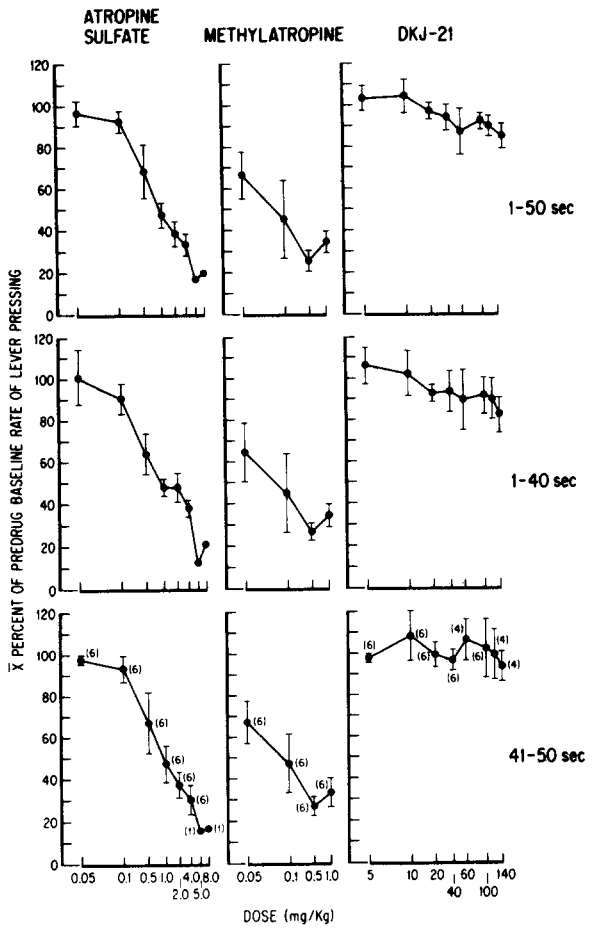

FIG. 3. Percent of predrug baseline rate of responding following presession injection of atropine, methylatropine and DKJ-21. The results are presented separately for the entire 50-sec interval, the first 40 sec (1–40 sec) and the last 10 sec (41–50 sec) of tte interval. Each bar represents the mean±SEM for all animals studied. The number of rats tested are indicated within the bars.

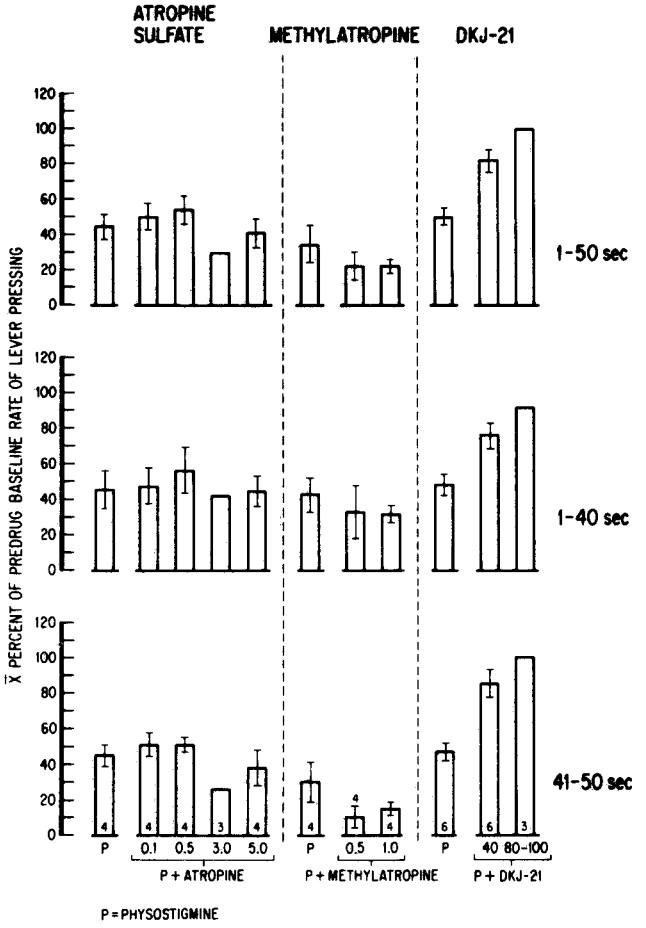

FIG. 4. Percent of predrug baseline rate of responding following injection of physostigmine alone and physostigmine plus atropine, methylatropine, or DKJ-21. Each bar represents the mean±SEM. The numbers within each bar represent the number of animals studied. The dose of physostigmine in each subject was adjusted to produce approximately equivalent effects and ranged between 250 and 600 μg/kg.

peripheral effect. This hypothesis is supported by the finding that partially chewed food pellets were left in the food bin and excreta tray during administration of both drugs. A primary effect of antimuscarinic drugs is a decrease in salivary secretions. Since it is difficult to chew and swallow food with a dry mouth, it is likely that the rats decreased response rates for lack of reward. This possibility previously has been suggested by others [13] and is supported by our finding that rats deprived of water for 24 hr also decreased their response rates and failed to eat completely all the food pellets. Since atropine and methylatropine produce similar peripheral effects it is likely that at least a portion of the behavioral inhibition produced by atropine also is mediated peripherally.

Neither atropine nor methylatropine prevented the reduction in responding produced by physostigmine, although in one of four rats atropine did reduce to some extent the suppressant effect of the cholinesterase inhibitor. In two other animals, the combination of physostigmine plus atropine suppressed response rates less than atropine alone. Findings for these animals agrees with reports from other laboratories which indicate that atropine antagonizes the suppressive effects of physostigmine [3, 17, 18] and oxotremorine [12]. Leander [12] allowed pigeons free access to water during the experiment. In our study, no effort was made to prevent the dry mouth caused by the antagonists. The presence of xerostomia may have prevented more obvious signs of antagonism (e.g., restoration of response rates). Vaillant [18] routinely administered methylatropine along with physostigmine to counteract the peripheral muscarinic effects of the cholinesterase inhibitor. This combination may well exert different effects than seen with physostigmine alone.

DKJ-21 is a selective antagonist of central muscarinic receptors. It blocks almost completely the centrally mediated tremors produced by oxotremorine at doses which do not produce mydriasis [5,6]. In addition, DKJ-21 blocks the centrally mediated pressor response to physostigmine at doses which do not inhibit the vasodepressor effect of acetylcholine [4].

Analogous results were obtained in the present study. DKJ-21 prevented the reduction in lever-pressing behavior caused by physostigmine. In contrast to atropine and

methylatropine, however, no partially chewed food pellets were observed during DKJ-21 administration, suggesting that this compound did not affect peripheral muscarinic receptors to reduce salivary secretions.

Of considerable interest is the observation that DKJ-21 did not influence responding when given alone. Suppression by physostigmine suggests that activation of endogenous cholinergic pathways inhibits food-maintained responding [18]. One would expect, therefore, that blockade of these pathways with antimuscarinic drugs should either have no effect or should increase, not decrease, these behaviors. No increase in responding was observed by any of the antimuscarinic drugs tested in this study. Instead, atropine evoked only a dose dependent decrease. If one assumes that the decrease in responding is due primarily to peripheral effects, as already noted, then the lack of inhibition by the centrally acting DKJ-21 is not surprising.

An alternate explanation for the inhibition of behavior produced by atropine is that any deviation from a set level of cholinergic activity will disrupt performance. In that case one would need to invoke a non-antimuscarinic action for the reversal of the effects of physostigmine by DKJ-21, since it does not disrupt behavior by itself at doses which antagonize physostigmine.

Relatively little work has been done with DKJ-21 regarding its receptor selectivity. It blocks the pressor effect of the selective ganglionic muscarinic agonist McN-A-343 [5,14] but does not inhibit the vascular effects of acetylcholine, noradrenaline or angiotensin [4]. The ganglionic muscarinic receptors are thought to resemble most closely central muscarinic receptors [9]. Muscarinic antagonists reduce brain acetylcholine levels and this property is shared by DKJ-21 [16]. The pressor response to physostigmine, which is mediated through central muscarinic receptors [2] also is blocked by DKJ-21. Unfortunately, however, the role of muscarinic blockade in these effects of DKJ-21 is circumstantial and an action on other neurochemical systems in the brain cannot be discounted.

In addition to pharmacologic properties of drugs per se, rate of responding [15] and degree of stimulus control [7, 10, 11] are important determinants of the behavioral effects of drugs. Yet, the role of these variables in the present studies was minimal at best. Both atropine and methylatropine suppressed response rates in each portion of the interval to a comparable extent whether or not food was signaled. The effects of physostigmine on behavior, and their antagonism by DKJ-21, also seemed independent of rate and stimulus control. Both atropine and methylatropine produced marked xerostomia. Since "dry mouth's produced by 24 hour water deprivation suppressed responses more in the first portion of the interval (low rates) than in the last portion (high rates), the opportunity to observe the influence of rate-dependency in the present studies was diminished.

Similarly, the present studies did not provide a satisfactory opportunity to assess the role of stimulus control. For five of the six animals, the control over response rates by temporal factors was much greater than the control exerted by the CS. While subtle effects of the CS could be measured, the "scalloped" response rate was relatively independent of the CS. Hence, it is likely that a true gradient of degree or strength of stimulus control was not achieved in the present study.

In summary, DKJ-21 is an effective antagonist of the rate-reducing effects of physostigmine. The antagonism was due to central effects, and DKJ-21 alone had little, if any, consistent effects on response rate. By virtue of its high degree of central selectivity and its effectiveness as a cholinesterase antagonist, DKJ-21 may have important advantages over atropine for studies of the role of central cholinergic systems in behavior.

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