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Influence of Atropine and N-Methyl Atropine Pretreatments on Behavioral and Physiological Effects of the Irreversible Muscarinic Agonist, BM123[1]

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OVERSTREET, D H , R. W. RUSSELL, R A BOOTH AND D J JENDEN *Influence of atropine and N-methyl atropine pretreatments on behavioral and physiological effects of the irreversible muscarinic agonist, BM123* PHARMACOL BIOCHEM BEHAV **26**(3) 475–481, 1987 – The irreversible muscarinic agonist, BM123 (63 μmoles kg^{-1}, IV), was shown to produce central and peripheral physiological signs characteristic of cholinergic agonists It also induced hypothermia, elevated nociceptive thresholds, reduced locomotor activity and disrupted spontaneous alternation performance in rats The centrally acting muscarinic antagonist, atropine (50 μmole kg^{-1}) prevented or reduced all the above effects of BM123 when given SC 40 min prior to the BM123 injection. In contrast, the peripherally acting muscarinic antagonist, N-methyl atropine, prevented only the peripheral effects and the elevated nociceptive thresholds Habituation of activity during a 20 min session was observed in all groups despite different levels of general activity These findings are consistent with a model in which atropine and N-methyl atropine compete with BM123 for reversible association with the muscarinic receptor. In the case of BM123 administered alone, the association results, first, in agonist effects and proceeds to form an irreversible complex Our present results show that by competing with BM123 for mAChR sites during the initial, reversible state of the interaction, atropine blocks the cholinomimetic effects of the agonist during both this state and its otherwise subsequent irreversible state

Atropine N-methyl atropine BM123 Drug interaction Muscarinic receptor Reversible association

REPORTS from our laboratory have presented evidence that BM123 (Fig. 1), an alkylating analog of oxotremorine, is a potent muscarinic agonist which binds selectively and irreversibly to the muscarinic receptor (mAChR) [6, 7, 14]. In aqueous solution at neutral pH BM123 forms an aziridinium ion, the active species of the compound, which initially binds reversibly to its receptor. Later, selective and irreversible reduction in the binding of [^{3}H](−) QNB (without significant effects on affinity) indicates a covalent and irreversible interaction of the aziridinium ion with the recognition site of the mAChR The effect is to decrease the density of functional receptors, return to normal functional densities being dependent upon *de novo* synthesis of mAChR The reactions involved may be represented as follows

$$A + R \rightleftharpoons AR \rightarrow AR^*$$

where A is the aziridinium ion; R, the mAChR; AR, the initial, reversible ion-receptor complex; and AR*, the irreversible alkylated complex. Cyclization of BM123 to form the aziridinium ion occurs relatively slowly; thus its administration resembles an infusion of the ion. BM123 is so selective for mAChR that it is almost inactive at nicotinic receptors. For these several reasons BM123 was chosen as the irreversible muscarinic agonist for the present research.

The series of experiments reported here was designed to study effects of BM123 on behavioral and physiological functions sensitive to manipulation of the cholinergic system when the density of available functional mAChRs was de-

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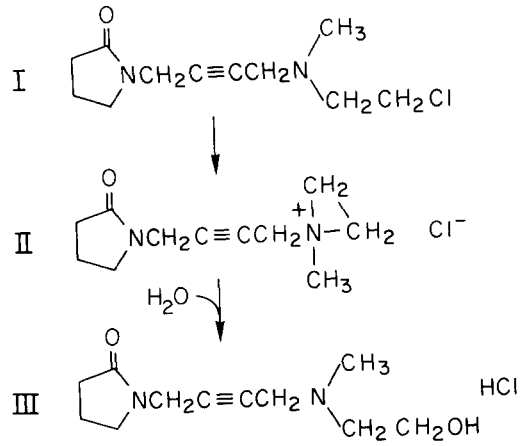

FIG 1 BM123, an analog of oxotremorine, which is converted to an aziridinium ion that selectively and irreversibly binds to the muscarinic receptor

creased competitively by pretreatment with the potent but reversible antagonist, atropine. The competition for receptor sites was arranged to occur during the reversible phase of the BM123-mAChR binding. To differentiate between central and peripheral effects of such pretreatment, N-methyl atropine, which does not readily penetrate the blood-brain barrier, was also included in the research design. *In vivo* and *in vitro* experiments in our laboratory have shown that, at adequate concentrations, atropine protects mAChRs in rat cerebral cortex from irreversible blockade by BM123 [6] We have also reported that N methyl atropine in 2 μM concentrations almost fully protects against the irreversible actions of BM123 in the isolated guinea pig ileum [14]. More recent experiments [22] have confirmed that *in vivo* pretreatment with atropine SC 30 min before injection of BM123 IV results in mAChR densities measured 3 hr later by [^{3}H](−) QNB binding which are not significantly different from the densities in tissues of rats administered atropine only. The results were the same for tissues from rat brain (cortex, striatum), heart and ileum. Ehlert and Jenden [7] have pointed out that atropine antagonizes the rate of alkylation of mAChRs in a manner that is consistent with competitive inhibition of the reversible step of the interaction. Therefore, in the present series of experiments it was predicted that pretreatment with atropine, having both central and peripheral actions, would significantly reduce or block all the behavioral and physiological effects of BM123 we reported in earlier experiments [17], while pretreatment with N-methyl atropine would influence only the peripheral effects of the compound

METHOD

Animals

Sprague-Dawley albino rats (240–260 g) were obtained from Simonsen Laboratories (Gilroy, CA) one week before the commencement of the experiments. They were housed individually in stainless steel cages under conditions of constant temperature (22±1°C) and humidity and a 12 hr light-dark illumination cycle They had free access to food and water throughout the experiments.

Pharmacological Treatments

BM123 was prepared by Dr Bjorn Ringdahl as previously described [14,21] Compounds such as BM123 spontaneously cyclize in aqueous solution at neutral pH and 37°C to form an aziridinium ion that does not readily penetrate the blood-brain barrier. For maximum stability stock solutions of BM123 were prepared in absolute ethanol at 0°C Fresh solutions from this stock were prepared every 10–15 min in normal saline solution at 0°C and administered in a single dose of 63 μmole kg^{-1} directly into the tail vein The dose level was chosen on the basis of preliminary experiments on dose effects and of other research in our laboratory [4] which showed that 63 μmole kg^{-1} reduced functional mAChRs to approximately 10% of normal Control animals were injected with saline Previous tests showed that ethanol in the concentrations involved (1 ml kg^{-1} of 2.3% solution) produced no significant effects on the variables measured [16,17]

Atropine and N-methyl atropine were obtained from Sigma Chemical Company (St. Louis, MO) Both compounds were dissolved in isotonic saline immediately before use They were administered SC in doses of 50 μmole kg^{-1} 40 min before injection of BM123. This dose level was selected on the basis of experiments by Waite [22] referred to earlier

Body Weight

Body weights were recorded during the course of the experiments using a Sartorius Capacity Balance Model 1404 MP8 and a Sartorius Model 7279 printer These measures served as indices of general health and of possible effects of pharmacological treatments on caloric intake

General Signs

The occurrence of three general signs of muscarinic activity were observed for 10 min starting immediately after the injection of BM123 or saline. Tremor was selected as a standard general indicator of central nervous system involvement, and chromodacryorrhea and salivation of peripheral involvement. The presence or absence of each was recorded and analyzed later by Fisher's Exact Test.

Core Body Temperature

Core body temperature was measured using a Bailey microprobe thermometer (BAT-12) providing readings to 0 1°C with near perfect stability RET-2 thermocouple probes inserted 6 cm into the rectum fed information into the thermometer The probes were of the copper/constantan type with fast response times They were small in physical size, facilitating insertion into the rectum Baseline temperatures were taken immediately prior to pretreatment with atropine, N-methyl atropine and saline and were recorded at 1 hr intervals for 4 hr after the challenge injections.

Nociception (Algesia)

Nociceptive thresholds were determined by the up-and-down procedure as developed in our laboratory [3]. Measurement of footshock, i.e , flinch and jump, thresholds involved placing the animal in a test chamber, the floor of which consisted of stainless steel rods through which electric shocks of varying intensity could be delivered Shock intensities were available from 0 05 to 4.0 mA in 20 steps arranged logarithmically Use of the full range of intensities was never necessary in determining thresholds. Each shock pulse (60 Hz) had a duration of 0 5 sec and shocks were delivered at 10

sec intervals. Shock levels at the start of an up-and-down series were set at midpoints of the ranges within which preliminary experiments had shown the thresholds likely to occur The experimenter then adjusted the intensity in accordance with the animal's response on each particular trial, i.e , raised 0 1 log unit when no response occurred and lowered 0 1 log unit when a response had been made. A "flinch" was defined as elevation of one paw from the grid floor and "jump" as rapid movement of three or four paws involving withdrawal from the floor. Thresholds were measured prior to the pretreatments and 1 hr after the challenge injections

Activity

General activity of the rats was measured in circular open field chambers with a diameter of 60 cm. The interior walls of the chambers were fitted with two sets of photocells and infrared-emitting diodes One set, 4 cm above the floor, measured horizontal (locomotor) activity, while the second set, located 12 cm from the floor, concurrently measured vertical (rearing) activity The floor was composed of a fine screen wire mesh, and the chambers were covered with plywood tops Red light (50 watt bulb) illumination and white noise were used to maintain constant conditions of visual and auditory stimulation. The activity chambers were interfaced with a TRS-80 Model III microcomputer, which automatically recorded all light beam breaks and, at the end of each animal's 20 min session, printed the results in terms of horizontal and vertical movements Totals for the 20 min were taken as the basic measures of activity Pretreatment baselines were not measured so that habituation following the BM123 or saline challenges could be studied (see following paragraph)

Habituation

By sampling activity as a function of time in the open field situation, it was possible to obtain information about habituation, a type of non-associative learning. Heise [9] has defined habituation as " . a primitive form of learning, since it constitutes an enduring behavioral change as a consequence of experience with the environment." It has been described as the most ubiquitous form of learning [8] and as more pragmatic than associative models in understanding many research results [1] Its relations to changes in transmitter release, receptor sensitivity and second messenger systems have been explored [10]. Within the present context "habituation" is used as a theoretical construct, while its observable correlates are operationally measurable in terms of decrements in behavioral responding during a standard test period (see Peeke and Petrinovich [13] for a fuller development of this distinction)

Spontaneous Alternation

Spontaneous alternation, selected as an index of memory [9,23], was measured in a T-maze The arms of the T were 13 cm wide and 30 cm deep A 40 cm starting alley was separated from the choice point between the other two arms by a guillotine door. A stainless steel plate provided a floor for the maze in order to facilitate cleaning between trials A single session of spontaneous alternation was given 48 hr after the BM123 injection The session consisted of two trials on the first, forced run, one of the alleys was blocked and the rat could traverse the open alley only, on the second choice trial, both alleys were available for selection. A trial terminated when the animal's four paws were in the selected alley. If, during a choice trial, an animal selected the previously blocked alley, an alternation response was recorded Rats were given 60 sec to move into the open alley on the forced run and then pushed gently into it. There were 30 sec between the forced and choice runs. They had 90 sec to select one of the alleys on the choice run. Those not responding in 90 sec were not used in the analyses

Procedure

The experimental design involved four basic phases During Phase 1 (1 week) the animals were adapted to the general laboratory conditions. In Phase 2 they were pretreated with isotonic saline, atropine or N-methyl atropine administered SC Phase 3 occurred 40 min later when saline or BM123 was injected IV. All injection volumes were 1 ml kg^{-1}.

Behavioral and physiological variables were measured during Phase 4. As described earlier, some variables were recorded repeatedly Others were measured during one assay only activity and habituation 24 hr and spontaneous alternation 48 hr after the second pharmacological treatment

Statistical Analyses

The general approach to statistical analyses of the research data was by one-way and two-way ANOVAs, the former for analyses of differences *among treatments* and the latter when the data also permitted analyses of repeated measurements Further tests for significance of changes over time were carried out *within* each treatment group using one-way ANOVAs for repeated measures when two-way ANOVAs indicated that significant trends existed When significant effects were found among treatments, ANOVAs were supplemented by Scheffe *post hoc* contrasts (Scheffe, 1953) In cases involving 2×2 contingency tables, Fisher's exact test was applied Significance is defined in terms of the 0.05 level of confidence, with exact p values noted in the text The time scores in the spontaneous alternation assay were not suitable for parametric analyses because scores were truncated at the high end, consequently, the nonparametric Kruskal-Wallis one-way ANOVA was used.

RESULTS

In a preliminary set of analyses, animals that were pretreated in Phase 2 with saline, atropine or N-methyl atropine and challenged in Phase 3 with saline only were compared as a means of observing possible carryover effects of the pretreatments. In none of the behavioral and physiological variables measured during Phase 4 was there evidence for such effects. No effects on general signs were observed One-way ANOVAs provided the following F-values core body temperature, $F(2,5)=3.73$, $p=0.10$, nociception· flinch, $F(2,5)=1.44$, $p=0.67$; nociception jump, $F(2,5)=0\ 46$, $p=0.66$ The results of these groups were, therefore, combined into a single "reference group" to which we could compare the other three treatment groups· saline-BM123, atropine-BM123 and N-methyl atropine-BM123.

As would be expected, when animals were assigned randomly to the various treatment groups, pretreatment baseline measures were comparable.

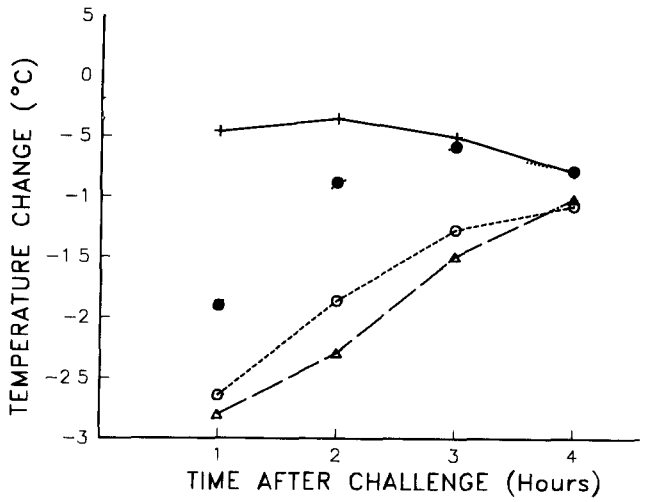

FIG 2 Differences between pretreatment core body temperatures (°C) and temperatures at various times after challenge IV injections of BM123 (63 μmoles kg^{-1}) or saline The pretreatments were given 40 min prior to the challenge injections (+) saline-saline (N=7), (●) atropine-BM123 group (N=6), (○) saline-BM123 group (N=7), (△) N-methyl atropine-BM123 group (N=7)

Body Weight

No animal exhibited dramatic changes in body weight during the three-day experiment. The reference and atropine-BM123 groups showed small increases in body weight, +10.3±2.2 and +6.8±1.2 g, respectively On the other hand, the saline-BM123 and N-methyl atropine-BM123 groups showed small decreases in body weight, −4 3±3 0 and −5.7±2.2 g, respectively One-way ANOVA of these data indicated that these weight changes were significantly different, $F(3,13)=12.28$, $p=4.3\times10^{-4}$. Scheffe contrasts established that the increases in body weight shown by the reference and atropine-BM123 groups were not significantly different from each other, but were significantly different from the decreases shown by the saline-BM123 and N-methyl atropine-BM123 groups Thus, pretreatment with atropine but not N-methyl atropine prevented the effects of BM123 on body weight.

Signs

Effects of the various treatments on signs are fully predictable from knowledge of the actions of the various compounds involved [4,17] All animals in the saline-BM123 group showed tremor, chromodacryorrhea and salivation, the N-methyl atropine-BM123 combination produced tremor in all rats, but no peripheral signs; and none of the reference and atropine-BM123 animals were overtly affected. Fisher Exact Tests showed that the saline-BM123 and N-methyl atropine-BM123 groups were significantly different from the reference group ($p=2.9\times10^{-4}$) for tremor, while the atropine-BM123 group was not ($p=1.0$). Only the saline-BM123 group was significantly different ($p=2.9\times10^{-4}$) from the reference group for the peripheral signs

Core Body Temperature

The effects of the various treatments on core body temperature are illustrated in Fig. 2. Two-way ANOVA established that there were significant treatment and time effects, $F(3,24)=8.42$, $p=5.3\times10^{-4}$; $F(3,72)=33.75$, $p=8.0\times10^{-10}$, respectively. There was also a significant interaction, $F(9,72)=8.64$, $p=1\ 0\times10^{-8}$, between treatments and time, indicating that the effects of the various treatments differed as a function of time after injection

Pretreatment with atropine partially prevented effects of the BM123 challenge and resulted in faster recovery to control levels than did pretreatments with N-methyl atropine or saline (see Fig 2). At 1 hr after injection one-way ANOVA supported by Scheffe contrasts showed that all treatment combinations induced significant hypothermia when compared with the reference group

One-way ANOVA supported by Scheffe contrasts of the temperature data at 2 hr after the BM123 injections confirmed that the atropine-BM123 group recovered more rapidly, $F(3,28)=17.43$, $p=3\ 53\times10^{-6}$. There were no significant differences between the reference and atropine-BM123 groups or between the saline-BM123 and N-methyl atropine-BM123 groups, but the two former groups were significantly different from the two latter groups Thus, atropine pretreatment provided some "protection" against the hypothermic effects of BM123, whereas N-methyl atropine pretreatment did not

Nociception (Algesia)

As measured by our method, nociceptive thresholds are very consistent. In testing for significant differences between treatment groups, it was decided to use each animal as its own control, i e , carry out analyses in terms of differences, Δ, between measures for each animal at baseline and after challenge treatments ANOVA showed no significant effects of the various treatments on flinch thresholds, $F(3,34)=1.74$, $p=0\ 19$ In fact, all treatments had very little influence on them, as shown in Table 1

By contrast, jump thresholds were differentially affected, $F(3,24)=6\ 41$, $p=2.4\times10^{-3}$. Scheffe contrasts confirmed the impression in Table 2 that the saline-BM123 group exhibited significant hypoalgesia when compared with the other three treatments. The latter were not significantly different from each other Thus, both atropine and N-methyl atropine blocked the effects of BM123 on jump thresholds

General Activity

Results of the various treatments on locomotion and rearing are summarized in Table 2 ANOVA for locomotion, $F(3,24)=39\ 16$, $p=2\ 3\times10^{-9}$, established that there were highly significant differences in between-treatment effects *Post hoc* analyses using Scheffe contrasts showed that all treatment groups were hypoactive in comparison with the reference controls All these groups had been challenged with BM123 Animals pretreated with saline or with N-methyl atropine were significantly more hypoactive than those pretreated with atropine, but not from each other These results indicate that the atropine provided at least some central protection against the reduction in activity produced by BM123

ANOVA for among-treatment effects confirmed the significance of trends that are apparent in Table 2, i e., activity in the vertical dimension was similarly affected, $F(3,24)=40.95$, $p=1.7\times10^{-9}$ Scheffe comparisons demonstrated that all treatments involving BM123 challenges

TABLE 1

EFFECTS OF THE VARIOUS EXPERIMENTAL TREATMENTS ON NOCICEPTIVE THRESHOLDS

		mA Difference From Pretreatment (Δ)			
		Flinch		Jump	
Treatment	n	Mean	SEM	Mean	SEM
Saline-Saline	8	+0 011	0 008	+0 008	0 021
Saline-BM123	7	+0 040	0.014	+0 129	0 031
Atropine-BM123	6	+0 013	0 012	+0 024	0 016
N-methyl atropine-BM123	7	+0 020	0 010	+0 020	0.015
	N=28				

TABLE 2

EFFECTS OF THE VARIOUS EXPERIMENTAL TREATMENTS ON GENERAL ACTIVITY

		Locomotion*		Rearing†	
Treatment	n	Mean	SEM	Mean	SEM
Saline-Saline	8	949	61 4	302	15 4
Saline-BM123	7	283	40 5	66	16 2
Atropine-BM123	6	605	55 8	202	26 2
N-methyl atropine-BM123	7	335	37 4	65	17 7
	N=28				

*Total number of line crossings during 20 min assay
†Total number of rearings during 20 min assay

produced significant hypoactivity relative to reference control levels. Again, animals in the atropine-BM123 group were partially protected against effects of BM123, i.e., were significantly less hypoactive than the other two groups.

Habituation

The three experimental treatments produced similar effects on the processes of habituation in the horizontal (locomotion) and vertical (rearing) planes. Two-way ANOVA of measures for locomotion showed that there were significant differences among treatments, $F(3,24)=38\ 91$, $p=1.5\times10^{-9}$, within treatments, $F(9,216)=54\ 52$, $p=4.0\times10^{-10}$, and with a significant interaction factor, $F(27,216)=2\ 27$, $p=6.5\times10^{-4}$. As illustrated in Fig 3, the significant interaction factor may well be a function of the truncated measuring scale, i.e., it was not possible for scores to be less than zero Therefore, those groups with lower initial scores could not exhibit as large decreases in activity In any case, all four groups showed habituation as the 20-min assay period continued One-way ANOVAs within each group indicated that the habituation was highly significant for all [reference, $F(9,63)=22.54$, $p<10^{-10}$, saline-BM123, $F(9,54)=11.93$, $p=2\ 0\times10^{-10}$, atropine-BM123, $F(9,45)=8\ 70$, $p=3\ 2\times10^{-7}$, N-methyl atropine-BM123, $F(9,54)=15.56$, $p<10^{-10}$].

The patterns of habituation in the vertical plane were similar to those observed for the horizontal plane, so a figure is not presented Two-way ANOVA of the data for rearing

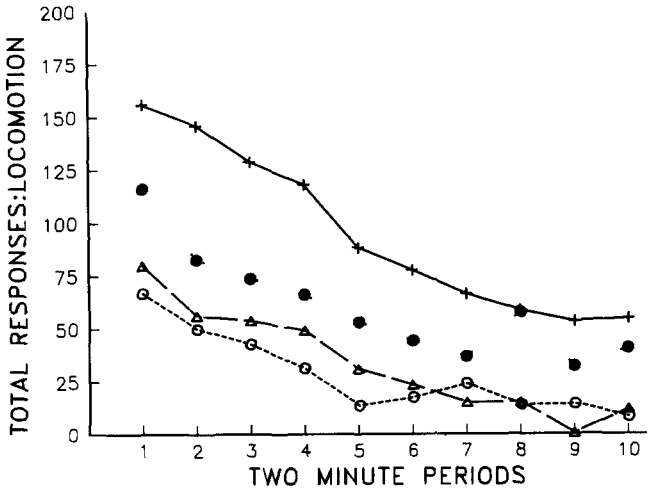

FIG 3 Habituation of locomotor activity 24 hr after challenge IV injections of BM123 (63 μmoles kg^{-1}) or saline in rats pretreated SC with saline, atropine (50 μmoles kg^{-1}) or N-methyl atropine (50 μmoles kg^{-1}) See legend to Fig 2 for code

established that there were significant differences between, $F(3,24)=42\ 09$, $p=1.4\times10^{-9}$, and within, $F(9,216)=8.33$, $p<10^{-10}$, treatments, but the interaction factor was not significant. All treatments showed significant habituation as evidenced by one-way ANOVAs within each treatment [reference, $F(9,63)=3.07$, $p=4\ 7\times10^{-3}$, saline-BM123, $F(9,54)=5\ 10$, $p=5.4\times10^{-5}$, atropine-BM123, $F(9,45)=2\ 89$, $p=0\ 01$, N-methyl atropine-BM123, $F(9,54)=4\ 84$, $p=9\ 4\times10^{-5}$]

Spontaneous Alternation

Observations of spontaneous alternation, recorded during assays 48 hr after the challenge injections, provided three measures. number of animals alternating, time (sec) taken to complete the forced trial, and time taken to complete the choice trial. For the first variable comparisons between groups were evaluated using Fisher's Exact Test These analyses gave results similar to analyses of other variables described above there were no significant differences between the reference and atropine-BM123 nor between the saline-BM123 and N-methyl atropine-BM123 groups However, the numbers of animals alternating under the two former conditions (83%) was much greater than in the latter two groups (36%) Fisher's Test showed the difference to be statistically significant at $p=0\ 03$ Thus, the central effect of atropine preatment was to protect against the impairment induced by the BM123 challenge

More animals from the saline-BM123 and N-methyl atropine-BM123 treatment groups required gentle pushing on the forced trials the median values for each of these groups was 60 sec. In contrast, the median values for the reference and atropine BM123 treatment groups for the forced trials were 20 and 8 sec, respectively Kruskal-Wallis one-way ANOVA on these data indicated a significant difference, $H(3)=7\ 88$, $p<0.05$ The times to complete the choice trial (to alternate) were more comparable among the groups, with median values of 5, 10, 6 and 7 sec for the reference, saline-BM123, atropine-BM123 and N-methyl atropine-BM123 groups, respectively. Kruskal-Wallis one-way ANOVA on these data indicated no significant difference, $H(3)=4\ 17$, $p>0\ 20$

DISCUSSION

The present experiments were designed to study effects of a unique muscarinic agonist on behavioral and physiological variables considerably distal to the agonist-receptor complex itself. BM123 forms its active species, an aziridinium ion, relatively slowly after entering the body. Ringdahl *et al* [14] have reported the time parameters in the formation and decomposition of this ion. The rate constant for cyclization of BM123 to its aziridinium ion was found to be 1.14 hr^{-1} at pH 7 0 and 37°C and that for decay of the ion, 1.98 hr^{-1}. The ion concentration reached its maximum after 40 min. The aziridinium ion rapidly forms reversible complexes, AR, with affinity sites on the mAChR and these, in turn, slowly convert to covalent complexes, AR* [7]. Because the rate constant for AR-AR* is 0 02–0 03 min^{-1}, effects lasting more than 1–2 hr must be due to the AR* and not the AR state Muscarinic antagonists, e.g., N-methyl atropine [14] and atropine [7], have been shown to prevent the alkylation of mAChRs Results of present studies are discussed within this framework, emphasizing the cholinomimetic effects of BM123, the effects of BM123 in its reversible and irreversible states, central versus peripheral effects of pretreatments with muscarinic antagonists and the extent to which antagonists "protect" against effects of the agonist

Cholinomimetic Effects

Comparisons of the saline-BM123 and the reference groups show that BM123 acted as a muscarinic agonist Evidence of hyperstimulation appeared in physiological variables as tremor, chromodacryorrhea, excessive salivation and hypothermia Sensory and perceptual processes, as reflected in nociceptive (jump) thresholds, were impaired. General activity, both locomotion and rearing, was suppressed Cognitive processes as represented by memory in the spontaneous alternation situation were also impaired. These results are consistent with our earlier findings [12,17]

Effects of BM123 in Its Reversible and Irreversible States

As indicated earlier, the rate constant for AR-AR* is such that effects lasting more than 1–2 hr must have been related to the irreversible AR* while those occurring within a shorter time frame may be related to the reversible AR complex All physiological variables measured had fully recovered or were well on the way to the reference group levels by the end of the second hour after injection of BM123 The nature of the behavioral measures precluded repeated assays and, therefore, recovery times could not be estimated Nevertheless, the results show that significant behavioral effects of BM123 were apparent up to 48 hours after the injection, i e , at a time when only the irreversible AR* state would have existed

Central vs Peripheral Effects

Results of our earlier experiments [17] had indicated that injection of BM123 IV produced effects identifiable as involving central and/or peripheral sites of action The studies by Waite [22] had shown that both such effects could be prevented by pretreatment with atropine Inclusion of the quaternary antagonist, N-methyl atropine, made it possible to differentiate between central and peripheral effects of hyperstimulation by BM123 on the behavioral and physiological variables measured

The differential effects of the two antagonists are clearly seen in the measures of tremor, chromodacryorrhea and salivation These parameters had been selected because of the well-documented involvement of central cholinergic processes in tremor and of peripheral cholinergic processes in the other two signs Atropine affected the action of BM123 at both sites, while N-methyl atropine had selective influences on peripheral sites only These clear cut differences substantiate the utility of using the tertiary and quaternary antagonists in these experiments

The maintenance of a constant body temperature requires a balance between heat production and heat loss, both of which involve central and peripheral mechanisms Neither atropine nor N-methyl atropine significantly blocked the hypothermia induced by BM123 at the one hour time point (Fig. 2), but the atropine-pretreated animals recovered more rapidly This finding indicates that at least part of BM123's hypothermic effects are due to hyperstimulation of central muscarinic receptors

Nociception, as measured, is dependent upon both pe-

ripheral (sensory input) and central (perceptual and motor output) processes [2]. The fact that both antagonists completely prevented BM123's effects on jump thresholds suggests that hyperstimulation of peripheral cholinergic receptors was primarily responsible for the increase in this measure induced by BM123.

General activity, initiated by the animal itself, has an obvious central component, which was influenced by atropine, but not by methyl atropine On the other hand, habituation, a central non-associative learning process, ran its course under all treatment conditions, despite different levels of activation. This result is consistent with other literature suggesting a lack of involvement of cholinergic processes in habituation of locomotor activity [11,15] Finally, the fact that atropine-pretreated rats alternated at high rates whereas methyl atropine-pretreated rats did not indicates that BM123's influence on cognitive processes is predominantly a consequence of its interaction with central mAChRs (see [12,19]).

Extent of "Protection"

Results of the present experiments make it quite clear that pretreatment with atropine provides protection against the hyperstimulatory actions of BM123 in all of the major variables measured It is also clear that the extent of "protection" under the circumstances of the present experiments must be considered as a continuum and not a matter of all-or-none. For example, atropine pretreatment provides some protection against the hypothermic effects of BM123, as revealed by the more rapid recovery of this group (Fig. 2) The extent of "protection" may be related to the density of "free" or functional mAChRs, as suggested in our earlier report [17]

In summary, the present findings are consistent with a model in which atropine and N-methyl atropine compete with BM123 for reversible association with the muscarinic receptor. In the case of BM123 administered alone, the association results, first, in agonist effects and proceeds to form an irreversible complex associated with long term resistance to muscarinic stimulation [16] Our present results show that by competing with BM123 for mAChR sites during the initial, reversible state of the interaction, atropine blocks the cholinomimetic effects of the agonist during both this state and its subsequent irreversible state

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