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Journal
Psychopharmacologia, 20(4)

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Publication Date
1971

Peer reviewed

Psychopharmacologia (Berl.) 20, 366—382 (1971)

A Biphasic Action of Central Cholinergic Stimulation on Behavioral Arousal in the Rat*

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Received November 10, 1970
Final Version: January 25, 1971

Abstract. The effect of the cholinomimetic, pilocarpine, on behavioral arousal as measured by locomotor activity was investigated in the rat. Pilocarpine first produced a period of behavioral inhibition, the intensity and duration of which was dose-related. After the inhibitory phase, a period of marked psychomotor excitation was observed. Pretreatment with scopolamine prevented both the inhibitory and excitatory effects of pilocarpine. Scopolamine administered at the onset of the rebound hyperactive period, however, significantly potentiated this excitatory phase. The anticholinesterase, physostigmine, also had a biphasic effect on behavioral arousal. The results are interpreted as indicating induction of central adrenergic activity in response to central cholinergic stimulation.

Key-Words: Locomotor Activity — Body Temperature — Pilocarpine — Physostigmine — Scopolamine — Rebound.

Introduction

The cholinomimetic, pilocarpine, in accordance with other compounds which increase central cholinergic activity has been shown to induce a state of generalized response suppression in a variety of experimental situations. The inhibitory effect during cholinergic predominance has been observed in rats in conditioned avoidance responding (Pfeiffer and Jenney, 1957; Rosecrans, Dren, and Domino, 1968), in self-stimulation of the brain (Domino and Olds, 1968; Olds and Domino, 1969; Stark *et al.*, 1968), on various reinforcement schedules (Pradhan and Dutton, 1970),

* Supported in part by United States Public Health Service Grant MH-8501 from the National Institute of Mental Health to Byron A. Campbell. The authors gratefully acknowledge the encouragement and helpful criticism of Byron A. Campbell throughout all phases of this research.

and in locomotor activity (Fibiger, Lytle, and Campbell, 1970; Pradhan and Dutton, 1970). A cataleptic state in the mouse during central cholinergic stimulation has also been reported (Zetler, 1968). These findings can be accommodated by Carlton's (1963) theory which postulates an adrenergic basis to behavioral arousal which is antagonized by a reciprocally acting cholinergic system. Within this framework, a generalized trophotropic state characterized by immobility would be predicted during central cholinergic predominance.

In a recent experiment Manto (1967) reported that epinephrine-induced decreases in psychomotor excitation could be antagonized by scopolamine but not by its peripherally-acting analogue methylscopolamine. This was interpreted to indicate that adrenergic stimulation served to increase central cholinergic activity which was reflected in the decreased locomotor activity. This is perhaps the only example at a gross behavioral level of the well-documented rebound or autonomic induction phenomenon (Gellhorn, 1957). One example of this phenomenon at the level of the autonomic nervous system is that the pressor response to intravenously injected norepinephrine is frequently followed by a lesser depressor phase.

The present experiments were designed to examine the possibility of inducing rebound effects in response to central cholinergic stimulation. It was hypothesized that the generalized state of behavioral inhibition known to occur during central muscarinic predominance would be followed by a period of psychomotor excitation. In addition, insofar as the examples of induction cited above were produced by peripheral stimulation (epinephrine and norepinephrine do not readily pass the blood-brain barrier) it was of interest to determine if this phenomenon could be demonstrated in the central nervous system in the absence of peripheral cholinergic stimulation.

Methods

Sprague-Dawley male rats (Perfection Breeders, Douglassville, Pa.) weighing 250—275 g at the beginning of experiments were used in these experiments. Locomotor activity was measured in six cylindrical wire mesh cages (14 inches in diameter and 18 inches high). Two photocells were mounted on each cage one inch off the floor at 90 degrees to each other with their beams crossing in the center of the cage. The apparatus was programmed such that each time a photobeam was interrupted, a count registered on a print-out counter which printed the number of photobeam crossings at six minute intervals. Each activity cage was housed in a fan-ventilated wooden box. These boxes were dark except for the light given off by the photocells. Temperature ranged from 22—23°C. The rats had free access to food (Purina rat chow) and water

except during the time in the activity cages. In another experiment, a Yellow Springs Telethermometer (Model 43 TA) and appropriate thermistor were used to measure rectal temperature.

Fresh solutions of drug in pyrogen-free 0.9% NaCl (saline solution) were prepared daily. All drugs were administered intraperitoneally. The following drugs were employed: pilocarpine nitrate, physostigmine sulfate, methylscopolamine hydrobromide[1], and scopolamine hydrobromide. All dosages refer to the weight of the salt, and all drugs were injected to a volume of 1 ml/kg. Rats were injected with methylscopolamine hydrobromide (1.0 mg/kg) to antagonize any peripheral muscarinic effects. In pilot experiments this dosage of methylscopolamine did not have a significant effect on activity when compared to the effects of saline injection. Furthermore, observation of the animals indicated that this amount of methylscopolamine was sufficient to block the peripheral effects (e.g. salivation) of pilocarpine and physostigmine in the dosages used in these experiments.

Experiment I

The purpose of this experiment was to examine the effect of various dosages of pilocarpine on behavioral arousal as measured by locomotor activity in a photocell cage. Rats were injected with methylscopolamine HBr (1.0 mg/kg). Five minutes later they were challenged with pilocarpine nitrate (2.5, 5.0, 10.0, or 20.0 mg/kg) or saline and then immediately placed in the photocell cages for a 3 h period. The effect of various dosages of pilocarpine after methylscopolamine on locomotor activity is seen in Fig. 1. A two-way analysis of variance indicated that both the dosage effect ($F = 6.80$, $df = 4, 210$; $p < 0.01$) and the effect across time ($F = 17.52$, $df = 5, 210$; $p < 0.01$) were significant. The dosage X time interaction was also significant ($F = 6.01$, $df = 20, 210$; $p < 0.01$). It is evident that the higher doses of pilocarpine (10 and 20 mg/kg) produced an initial inhibitory effect on locomotor activity. A one-way analysis of variance on the total activity of the five groups during the first half hour indicates that there was a significant drug effect during this period ($F = 14.81$, $df = 35, 4$; $p < 0.01$). Both the 10 mg/kg group and the 20 mg/kg group showed significant inhibition compared to the saline group during this period (10 mg/kg: $t = 3.63$, $df = 14$, $p < 0.01$; 20 mg/kg: $t = 4.67$, $df = 14$, $p < 0.01$). Lower doses of pilocarpine failed to exert a significant inhibitory effect during this period (2.5 mg/kg: $t = 0.19$, $df = 14$, n.s. 5.0 mg/kg: $t = 1.02$, $df = 14$, n.s.).

Fig. 1 also indicates that with the higher doses of pilocarpine, a period of marked hyperactivity followed the initial inhibitory effect of

1 The Up John Company, Kalamazoo, Michigan made generous donations of methylscopolamine hydrobromide.

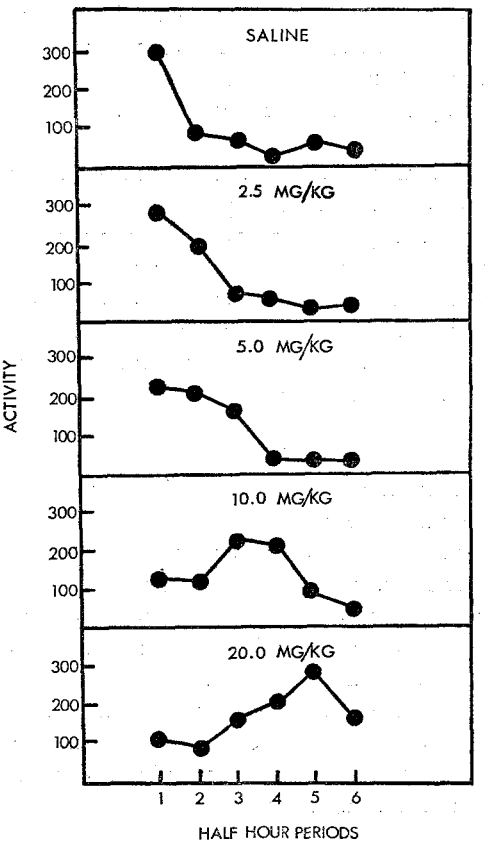

Fig. 1. The influence of various doses of intraperitoneally administered pilocarpine and methylscopolamine on photocell cage activity of naive rats for 3 h following injection

the drug. In the 10 mg/kg group the peak hyperactive period occurred during the third half-hour post-injection period. This effect was statistically significant ($t = 3.76$, $df = 14$, $p < 0.01$). With the 20 mg/kg group, the inhibitory phase appeared to persist longer than in the 10 mg/kg group and the peak hyperactive period did not occur until the fifth-half-hour post-injection period. It is noteworthy that while the 5 mg/kg group did not show a significant period of inhibition on the first half hour measure, this group was significantly more active than the saline group at 1 h ($t = 2.97$, $df = 14$, $p < 0.02$) and at $1^1/_2$ h ($t = 3.09$, $df = 14$, $p < 0.01$). The lack of a significant inhibitory effect during the first half hour in this group was due to the fact that the inhibition induced by pilocarpine lasted less than one half hour (approximately 18 min during

which $t = 3.66$, $df = 14$, $p < 0.01$) and was obscured in the one half hour measure by the ensuing rebound hyperactivity which occurred during the remaining 12 min of that period. The 2.5 mg/kg group did not differ significantly from the saline group at any time during the 3 h session.

This experiment demonstrates that pilocarpine has a biphasic effect on locomotor activity. The initial response is the expected inhibitory action which has previously been observed in other behavioral tests. The second effect of pilocarpine, however, has not previously been reported and consists of an extended period of hyperactivity. This rebound period appears to occur only at those doses which elicit an initial inhibitory response. In addition the latency to rebound appears to be dose related insofar as it is increased with a higher dose of pilocarpine.

Experiment II

One possible explanation of the results of the previous experiment is that because of the inhibition induced by pilocarpine, the animals had no opportunity to explore the novel environment into which they were introduced. As the drug was metabolized or excreted, however, the animal began this exploratory behavior and that is what was reflected in the hyperactive phase following inhibition. This possibility was tested by first habituating the animal for one half hour to the photocell cage before administration of the drugs. After the habituation period each animal was injected with methylscopolamine (1.0 mg/kg) followed by pilocarpine (10 mg/kg) or saline 5 min later. Animals were then immediately returned to the photocell cages for a 2 h period. Six animals were run in each condition.

The results of this experiment are contained in Fig. 2. It is clear that essentially the same biphasic response to pilocarpine is observed in the habituated condition as in the unhabituated condition. As before, the most hyperactive phase occurred during the third post-injection half hour and during this period the drug group was significantly more active than the control ($t = 2.98$, $df = 10$, $p < 0.02$). Further evidence that the delayed excitatory action of pilocarpine was not simply exploratory behaviour which became manifest as the drug-induced inhibition was removed was also suggested from data in Experiment I. When the mean cumulative activity over the three hour period was plotted against dosage, cumulative activity was found to increase with increasing dosages of pilocarpine (Table). Contrary to these results, a delayed exploratory behavior hypothesis would predict that the total activity output over three hours would remain constant across dosages of pilocarpine.

These experiments indicate that pilocarpine has a biphasic effect on behavioral arousal which is not related to delayed exploratory behavior.

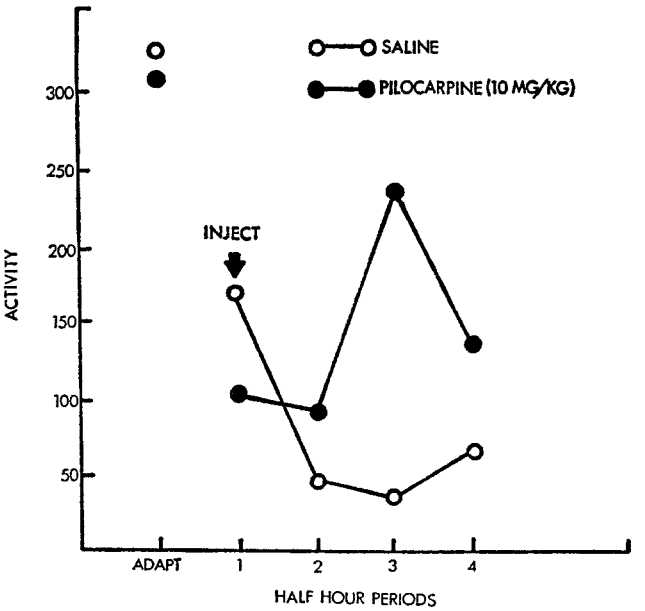

Fig. 2. Effects of pilocarpine or saline on the photocell cage activity of rats given 30 min of exploration prior to injection

Table. *Effect of various dosages of pilocarpine nitrate on total activity output over 3 h*

Pilocarpine dosage (mg/kg)	Mean cumulative activity	Pilocarpine dosage (mg/kg)	Mean cumulative activity
0	573	10.0	848
2.5	672	20.0	993
5.0	720		

The first phase, inhibition, is very likely a cholinergically-mediated effect insofar as many other experiments using a variety of drugs which increase central cholinergic activity have reported response inhibition (e.g. Pfeiffer and Jenney, 1957; Domino and Olds, 1968; Karczmar, Tongo, and Scotti de Carolis, 1970). The second phase, that of hyperactivity, may have several origins however. First, within Carlton's (1963) model of reciprocally acting central cholinergic and adrenergic mechanisms, it is possible that the effect of acute intense cholinergic stimulation was to induce a compensatory increase in central adrenergic activity. This can be considered as a homeostatic response which would serve to prevent excess cholinergic predominance. As the cholinomimetic drug was metabolized, cholinergic activity would decrease and it is at this time that the

compensatory excitatory adrenergic mechanism would become predominant. That is, the adrenergic system may be thought of as overresponding to the cholinergic stimulation, and it is this compensatory mechanism that is responsible for the rebound period.

A second possibility is that pilocarpine has an unspecified noncholinergic effect in the central nervous system. It may, for example, have central adrenergic properties which, although less potent than the cholinergic mechanism and therefore initially masked, are longer lasting and hence manifest themselves after the cholinergic property of the drug has dissipated. Although central adrenergic effects of pilocarpine have not been observed previously, this nevertheless remains a possibility. It is equally possible of course that pilocarpine causes psychomotor excitation through an unknown non-adrenergic mechanism.

A third possibility, related to the second, derives from experiments by Morgane (1969), Hernandez-Peon, Chavez-Ibarra, and Morgane (1962) and Smith (1970). Using direct chemical stimulation of the brain of cat, Morgane showed that both arousal and hypnogenic or sleep states could be elicited by cholinergic stimulation. He also reported that the sites responsible for these divergent behavioral results were in close proximity in subcortical areas of the brain and, in fact, overlapped in some loci. In addition, Smith (1970) has demonstrated increased behavioral arousal following direct cholinergic stimulation of the lateral hypothalamus in the rat. These results indicate that there are different cholinergic systems in the brain which are substrates for both behavioral inhibition and behavioral arousal. Within the context of the present experiments, it is possible that pilocarpine stimulates both of these systems. The inhibitory system may be prepotent initially but may, for example, metabolize pilocarpine more quickly than the excitatory system whose activity becomes dominant after the effect of pilocarpine has waned in the inhibitory system. The following two experiments were designed to discriminate between the above alternatives.

Experiment III

There are several reports which show that adrenergic arousal can be potentiated during central cholinergic blockade (Carlton, 1961; Carlton, 1963; Carlton and Didamo, 1961; Fibiger, Lytle and Campbell, 1970). In these experiments it was demonstrated that doses of anticholinergic drugs which by themselves had no effect on behavior, significantly potentiated the behavioral effect of amphetamine. Within this context, it was hypothesized that if the rebound period has adrenergic substrates, then a subthreshold dose of the central anticholinergic drug, scopolamine, should potentiate this period of arousal.

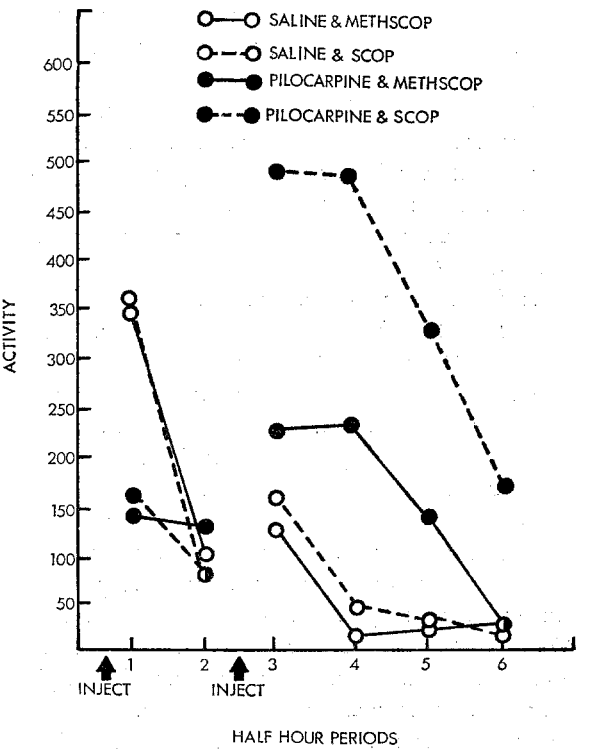

Fig. 3. The effects of scopolamine or methylscopolamine on the photobeam cage activity of rats given prior (by 90 min) injections of pilocarpine (filled circles) or saline (open circles). The first arrow labelled "inject" refers to the point at which saline or pilocarpine was administered, while the second arrow indicates the time at which the animals received scopolamine or methylscopolamine

The procedure was similar to that in Experiment I, except that animals were re-injected with either scopolamine or methylscopolamine at the beginning of the rebound period. Four groups were used: (1) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—saline—in photocell cage for 54 min—methylscopolamine hydrobromide (0.25 mg/kg); (2) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—saline—in photocell cage for 54 min—scopolamine hydrobromide (0.25 mg/kg); (3) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—pilocarpine nitrate (10 mg/kg)—in photocell cage for 54 min—methylscopolamine hydrobromide (0.25 mg/kg); and (4) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—pilocarpine nitrate (10 mg/kg)—in photocell cage for 54 min-scopolamine hydrobromide (0.25 mg/kg). With each group after

the last injection the animals were immediately replaced in the photocell cages for a further two hour period. Eight rats were used in each condition.

Fig. 3 contains the results of this experiment. First, it is clear that both pilocarpine groups were initially suppressed compared to the saline groups. The more significant feature of this experiment, however, is the finding that a subthreshold dose of scopolamine, but not its quartenary analogue methylscopolamine, produced a large potentiation of the rebound hyperactivity. A two-way analysis on the data during the last two hours indicated that there was a significant drug effect ($F = 38.67$, $df = 3, 112$; $p < 0.01$). The effect across time was also significant ($F = 12.75$, $df = 3, 112$; $p < 0.01$) as was the drug $\times$ time interaction ($F = 4.88$, $df = 9, 112$; $p < 0.01$). A comparison of the activity of the four groups during the second half hour after the last injection (half-hour period number 4 in Fig. 3) by one-way analysis of variance indicates that there was a large statistical difference between the groups during this period ($F = 26.60$, $df = 3, 28$, $p < 0.01$). Scopolamine at 0.25 mg/kg did not significantly increase the activity of the saline pretreated animals ($t = 1.63$, $df = 14$, n.s.). There was, however, a significant potentiation of the pilocarpine induced rebound by the same dose of scopolamine during this period ($t = 3.13$, $df = 14$, $p < 0.01$). Finally, it should be pointed out that in a separate experiment, saline injections instead of methylscopolamine in pilocarpine pretreated animals produced the same effect as methylscopolamine on the rebound activity. Unlike the centrally acting drug therefore, peripheral cholinergic blockade did not influence the period of behavioral arousal induced by pilocarpine.

This experiment indicates that pilocarpine does not stimulate both the excitatory and inhibitory cholinergic systems of Morgane. If this were the case the scopolamine administered at the beginning of the rebound period should block or decrease this hyperactive phase. Since the opposite results are obtained, this possibility cannot be entertained. These results are, however, consistent with the hypothesis that the rebound period is mediated by a central adrenergic mechanism. It is possible of course that subthreshold doses of scopolamine might potentiate some non-adrenergically medicated types of arousal, and so the present experiment cannot be regarded as a rigorous confirmation that the rebound period had adrenergic substrates.

Experiment IV

The purpose of this experiment was two-fold. First, it was hypothesized that if the inhibition elicited by pilocarpine was due to its effects on central muscarinic receptor sites, then pretreatment with scopolamine would attenuate this inhibition. Secondly, it should be possible to eluci-

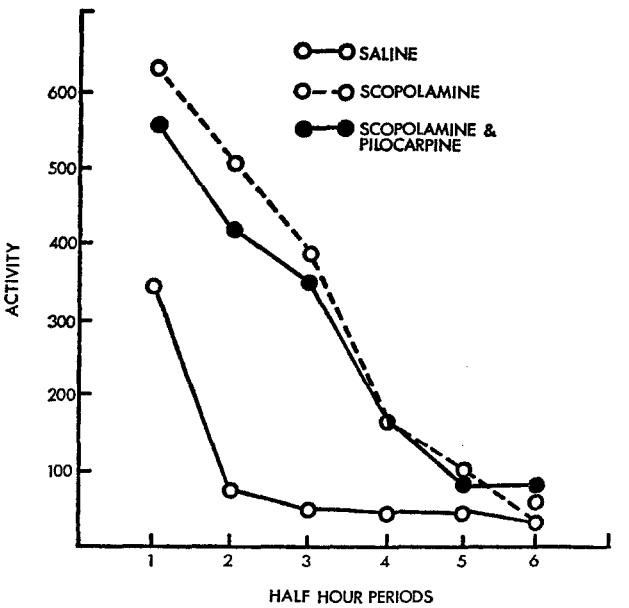

Fig. 4. The influence of scopolamine on the photocell cage activity of rats treated with pilocarpine or saline. Note that all injections were made immediately before the beginning of the activity test

date the nature of the rebound phenomenon by scopolamine pretreatment. As was pointed out above, rebound could be due either to increased activity in the adrenergic excitatory system in response to cholinergic stimulation, or it could be mediated by some other but as yet unknown non-cholinergic action of pilocarpine in the central nervous system. By pretreating rats with scopolamine before the pilocarpine administration it should be possible to distinguish between these two possibilities. It was hypothesized that if the rebound was a response to the initial inhibition produced by pilocarpine, then abolition of this inhibition by scopolamine should also prevent the rebound hyperactivity. If, on the other hand, pilocarpine has a non-cholinergic action in the central nervous system which underlies the rebound period, then scopolamine pretreatment should not affect the hyperactive period despite its attenuating action on the inhibition.

Three groups of rats were used: (1) saline—5 min—saline; (2) scopolamine hydrobromide (1.0 mg/kg)—5 min—saline; (3) scopolamine hydrobromide (1.0 mg/kg)—5 min—pilocarpine nitrate (10 mg/kg). After the last injection, the rats were immediately placed in the photocell cages for a 3 h period. Eight rats were run in each condition.

The results of this experiment indicate that scopolamine induced a state of behavioral excitation compared to the saline treated animals (Fig. 4). This effect of scopolamine has been noted previously (Pradhan and Roth, 1968; Campbell, Lytle, and Fibiger, 1969). In addition, scopolamine completely attenuated the inhibitory action of pilocarpine. This suggests that the inhibitory phase of pilocarpine action is due to central muscarinic stimulation. More important, however, is the effect of scopolamine pretreatment on the rebound phase. It is evident that rebound hyperactivity was not observed unless the initial inhibitory effect was induced first. The results of this experiment suggest, therefore, that the rebound period is in fact an excitatory phase occurring in response to the initial inhibition, and is not due to a non-cholinergic excitatory action of pilocarpine. If the latter had been the case, then scopolamine should not have prevented or influenced the rebound hyperactivity. In summary, this experiment is consistent with the hypothesis that it is the inhibitory phase which triggers the subsequent excitatory rebound phase.

Experiment V

The previous experiments have demonstrated that pilocarpine has a biphasic action on behavioral arousal. The purpose of this experiment was to determine if this phenomenon was the result of an idiosyncratic action of pilocarpine or if it could be elicited with another drug which increases central cholinergic stimulation. Physostigmine, through its inhibition of acetylcholinesterase, the enzyme responsible for the catabolism of acetylcholine, is known to increase central cholinergic activity.

As in the experiments with pilocarpine, all groups were pretreated with methylscopolamine to antagonize the peripheral effects of cholinergic stimulation. Two groups were run; (1) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—physostigmine sulfate (1.0 mg/kg); and (2) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—saline (0.9%). Immediately after the second injection *Ss* were placed in the photocell cages for a 3 h period. Eight rats were used in each condition.

The results of this experiment are seen in Fig. 5. A two-way analysis of variance indicated that the effect across time was significant ($F = 15.40$, $df = 5, 84$; $p < 0.01$), while the drug effect was not significant ($F = 0.08$, $df = 1, 84$; n.s.). The interaction effect, however, was significant ($F = 5.36$, $df = 5, 84$, $p < 0.01$). As with pilocarpine, the initial effect of physostigmine was to induce a period of behavioral inhibition. A comparison of the two groups during the first half hour indicates that this effect was statistically significant ($t = 3.09$, $df = 14$, $p < 0.01$). In addition, during the fifth half-hour period, the physostigmine group showed a significant increase in activity compared to the saline group

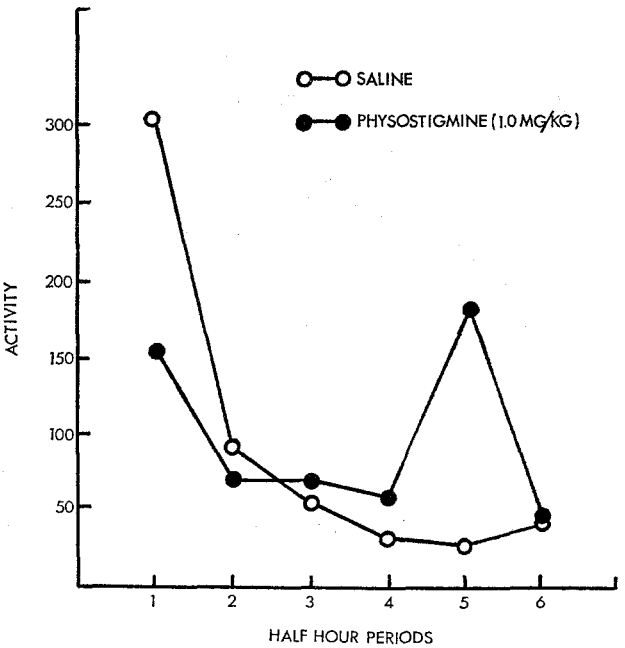

Fig. 5. Photocell cage activity of rats following injection with physostigmine or saline

($t = 3.19$, $df = 14$, $p < 0.01$). This was a short-lived effect which had subsided by the last 30 min period. These results indicate that the rebound phenomenon can also be obtained with physostigmine, a drug which increases central cholinergic activity by a mechanism substantially different from that of pilocarpine.

It is noteworthy, however, that while both pilocarpine (10 mg/kg) and physostigmine (1.0 mg/kg) induced approximately 50% inhibition compared to controls during the first half-hour period, the rebound hyperactivity with pilocarpine had a shorter latency and was of greater intensity and duration than that found with physostigmine. The reason for this difference is not known, but it may have been due to differences in the rate of metabolism between the two drugs. It is possible, for example, that pilocarpine is quickly metabolized or excreted from the brain and hence does not remain to antagonize the rebound hyperactivity. Physostigmine, on the other hand, may have a longer lasting cholinergic effect in the central nervous system which would tend to delay or obscure the rebound period.

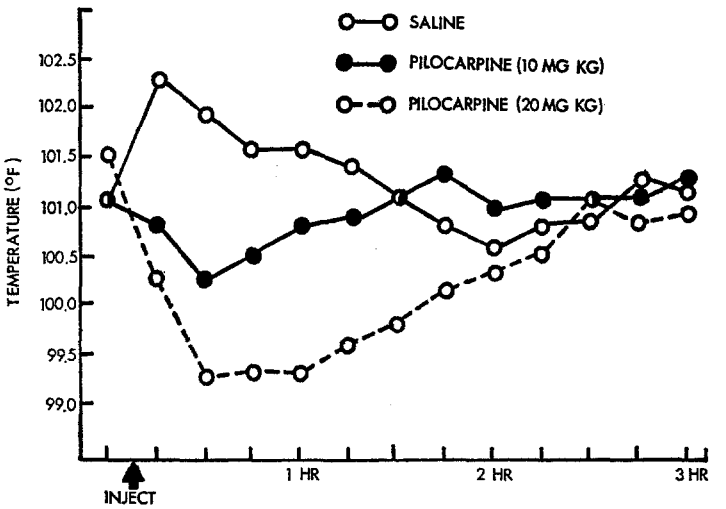

Fig. 6. The effects of two doses of pilocarpine or saline on the rectal temperatur of rats

Experiment VI

Pilocarpine, along with other cholinomimetic drugs, is known to have a hypothermic effect in rats and mice (Friedman and Jaffe, 1969; Lomax, Foster, and Kirkpatrick, 1969; Zetler, 1968). This effect can be easily blocked by scopolamine but not by methylscopolamine indicating that it is a central muscarinic response. Within the framework of the present experiments it was of interest to determine if the hypothermic response elicited by policarpine would be followed by a period of hyperthermia.

Each rat was handled for 10 min each day for 4 days during which the rectal temperature probe was inserted. To determine temperature, the thermistor probe was inserted 6 cm rectally and temperature was recorded 30 sec after the insertion. This was to adapt the rats to what might be a stressful and, therefore, temperature-contaminating procedure. On the fifth day, rats were randomly divided into one of three groups: (1) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—saline; (2) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—pilocarpine nitrate (10 mg/kg); and (3) methylscopolamine hydrobromide (1.0 mg/kg)—5 min—pilocarpine nitrate (20 mg/kg). Before the drug administration the rectal temperature of each animal was determined. Following the injections, temperature was recorded at 15 min intervals for 3 h. Eight rats were used in each condition.

The results of this experiment confirm previous reports that pilocarpine induces a state of hypothermia in the rat (Fig. 6). Of particular interest here, however, was the finding that temperature does not show the rebound effect seen with locomotor activity. Instead, pilocarpine caused a rapid fall in body temperature which slowly rose to control levels over the next 3 h. At no point was there any indication of hyperthermia following the period of lowered body temperature. It is evident, therefore, that the rebound phenomenon does not apply to all effects of the drug. Further research will ascertain which variables influenced by cholinergic drugs do and not do display this biphasic characteristic.

Discussion and Conclusions

The present studies demonstrate that pilocarpine and physostigmine procedure a dose-dependent biphasic action on behavioral arousal as measured by generalized activity. The first effect was a depression which was related to dosage in both intensity and duration; this phase was followed by a period of marked hyperactivity which lasted up to an hour.

It has long known that pilocarpine has a biphasic effect on blood pressure (Hunt, 1918). The initial period of hypotension is followed by a secondary blood pressure increase. However, the present results are not a reflection of these blood pressure changes. First, whereas the anticholinergic drug atropine immediately arrests the secondary hypertension after pilocarpine (Heaton and MacKeith, 1927; Root, 1951), in the present experiments anticholinergic administration potentiated the secondary hyperactivity. Second, adrenalectomy prevents the secondary pressure response to pilocarpine (Hunt, 1918; Heaton and MacKeith, 1927) but does not prevent the biphasic effect of pilocarpine on behavioral arousal (Fibiger and Lynch, unpublished observations). For these reasons, the phenomena are most likely unrelated.

The possibility that other peripheral changes might account for the present findings seems remote in view of the fact that moderate doses of scopolamine greatly influenced pilocarpine's effects on activity while its methylated analogue (which passes very poorly into brain) was without effect. Numerous other investigators have reported data showing that inhibition produced by cholinomimetics and anticholinesterases has central nervous system origins (cf. Carlton, 1963; Zetler, 1968). It seems most likely that the initial inhibitory phase is due to the action of pilocarpine and physostigmine on brain cholinergic synapses, presumably by stimulating receptor elements or prolonging the action of presynaptically released acetylcholine.

On the other hand, the rebound hyperactivity seen after the initial depression does not appear to be due to the continued action of pilo-

carpine in the brain. The possibility that pilocarpine stimulates a cholinergic behavioral excitatory system (Hernandez-Peon *et al.*, 1963) after its inhibitory effects have been reduced is negated by the study (Experiment III) showing that scopolamine administered at the end of the inhibitory period actually increased the subsequent hyperactivity. Of course, it is possible that pilocarpine has unspecified non-cholinergic (or possibly non-muscarinic) properties which become manifest after its cholinergically mediated depressive actions have been reduced. This, however, seems unlikely since our data (Experiment IV) show that scopolamine administered *with* pilocarpine blocked not only the initial inhibitory period but also the subsequent hyperactivity.

If the hyperactivity was due to non-muscarinic synaptic actions of pilocarpine, it is difficult to understand why they would be blocked by scopolamine. In summary, it seems unlikely that pilocarpine is directly responsible for the post-depression hyperactivity; furthermore, it appears that this "rebound" period is not dependent upon (and, in fact, is depressed by) central cholinergic systems.

Recent work showing that transmitter pools and synthesis rates are dependent upon levels on neural activity (e.g., Dairman and Udenfriend, 1970) suggest an explanatory mechanism for the "rebound" activity of the type discussed above. It seems plausible that the brain systems responsible for normally maintaining wakefulness might change their base level of functioning during prolonged antagonism by the inhibitory regions acted upon by pilocarpine. While the time-course of the inhibitory phase is short, it is certainly well within the range of times established for changing synthesis rates and pool sizes by chemical or electrical stimulation.

The nature of the transmitter system(s) responsible for the rebound hyperactivity is, of course, not known though acetylcholine is ruled out by the study (Experiment III) showing that scopolamine augments this period. Norepinephrine has long been assigned a role in the production of behavioral excitability by theorists (cf. Brodie, Spector and Shore, 1959) and several lines of evidence suggest that this adrenergic arousal system is inhibited by a cholinergic inhibitory system (Carlton, 1963; Fibiger, Lytle, and Campbell, 1970). Tentatively, then, we propose that the rebound hyperactivity reported above reflects a change in the level of functional synaptic activity in an adrenergic arousal system brought about by prolonged cholinergically mediated behavioral depression. The concept of antagonistic excitatory and inhibitory brain mechanisms which exist in a "homeostatic" state has received repeated consideration by neurophysiologists (Gellhorn, 1957; Hess, 1957) and electrophysiological examples of "rebound" phenomena have been reported (see Parmeggiani, 1968).

Whether "rebound" is found in all neurochemical systems or is unique to the example considered above remains to be established. It is interesting in this context to note that the hypothermia produced by pilocarpine (which is known to be a central effect; Friedman and Jaffe, 1969; Lomax *et al.*, 1969) is not followed by a rebound hyperthermia (Experiment VI); this is particularly relevant since antagonistic adrenergic and cholinergic systems have been shown to be involved in temperature regulation (Lomax *et al.*, 1969). The occurrence or non-occurrence of rebound-like phenomena, then, is not dependent solely on the drugs used or transmitter systems involved. Presently, however, there is little evidence concerning what additional variables might be involved.

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