Pharmacology Biochemistry and Behavior, Vol. 1, pp. 643 - 649. ANKHO International Inc., 1973. Printed in the U.S.A.

Cholinergic Influence on Intravenous Cocaine Self-administration by Rhesus Monkeys[1]

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(Received 20 September 1973)

WILSON, M. C. AND C. R. SCHUSTER. *Cholinergic influence on intravenous cocaine self-administration by rhesus monkeys.* PHARMAC. BIOCHEM. BEHAV. 1(6) 643–649, 1973.–The effects of intramuscular administration of atropine, methylatropine and physostigmine on intravenous cocaine self-administration in the rhesus monkey were ascertained. Atropine (0.5–2.0 mg/kg) increased cocaine intake, whereas methylatropine, over the same dosage range, produced no change in this behavior. Physostigmine (0.1–0.5 mg/kg) significantly depressed this behavior. The effect of atropine was interpreted as being the result of its central anti-cholinergic action and that of physostigmine, since it was opposite to that of atropine, was attributed to its central cholinergic action. Furthermore it was hypothesized that the effect of these cholinergic interactions on cocaine self-administration resulted from a modulation of the factors which may control self-administration i.e. drug-induced aversiveness or nonspecific behavioral disruption rather than any specific interaction with the neurochemical mechanisms of cocaine mediated reinforcement. The drug effects support the concept of a central cholinergic behavioral inhibitory system which when blocked, e.g., with atropine, results in behavioral activation.

Cocaine self-administration Psychomotor stimulant Atropine Physostigmine
Cholinergic behavioral facilitation Drug reinforcement Methylatropine

PREVIOUS investigations of intravenous cocaine self-administration in rhesus monkeys have demonstrated the daily stability of this behavior when the animals were permitted limited access e.g. 4hr; to the reinforcer. Several studies [12, 21, 14] have shown an inverse relationship between rate of cocaine-reinforced responding and the dosage of cocaine received per injection (unit dosage) over a wide range of unit dosages. The result of this relationship is that the amount of cocaine self-administered per day is fairly constant even though unit dosage may be altered several fold. Similar relationships have also been established with the psychomotor stimulants pipradrol, phenmetrazine and methylphenidate [21].

A previous investigation in rats has demonstrated the ability of the central anticholinergic agent atropine to potentiate the effects of d-amphetamine on continuous avoidance beahvior [6]. Qualitatively similar effects were also produced by equimolar dosages of methylatropine but the behavioral augmentation was much less. Therefore, the authors postulated that this drug effect resulted from central cholinergic blockage. Similar results have also been demonstrated with scopolamine [5]. Other investigators [18] demonstrated that atropine pretreatment potentiated the stimulant effects of cocaine and d-amphetamine on continuous avoidance behavior. Neither the dose of atropine nor the dose of the psychomotor stimulant (used in combination) when administered alone increased the frequency of the avoidance behavior above control rates. The effect of cocaine was potentiated more by the atropine than was that of d-amphetamine. Atropine methyl bromide produced similar results but only at dosages 35–60 times greater than that seen with atropine.

Other investigators have demonstrated in mice the ability of the central anticholinergic agents scopolomine and trihexyphenidyl, to potentiate the aggregated lethality effect of amphetamine [14]. This may have resulted from the ability of these compounds to peripherally suppress body heat dissipation by retarding sweating thereby augmenting amphetamine-induced hyperthermia. It has also been demonstrated that atropine facilitates the stimulatory actions of methamphetamine on intracranial self-stimulation behavior in the rat [19].

Since the results of these studies tend to implicate a facilitory effect of atropine on behavioral alterations produced by the psychomotor stimulants, one might expect an augmentation of the reinforcing efficacy of these compounds also. Therefore, the present study was conducted in an attempt to ascertain the effects of central cholinergic blockage on intravenous cocaine self-administration and more specifically to determine if atropine administration

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alters the reinforcing efficacy of cocaine. In addition, the data generated by this research may indicate the importance of central cholinergic processes in mediating cocaine-induced reinforcement.

METHOD

The animals in this study were four adult drug-naive male rhesus monkeys weighing 4.1 5.3 kg. Each animal was housed individually in an open-faced experimental cubicle containing a response lever and stimulus light and restrained by a stainless steel harness and arm assembly for the duration of the experiment [9]. The animals had free access to water and were fed twice daily at 8:00 a.m. and 4:00 p.m. After seven days of adaptation to this environment, chronic indwelling jugular catheters (I.D. = 0.81 mm, O.D. = 1.62 mm), constructed of siliconized rubber were surgically implanted under pentobarbital anesthesia (30 mg/kg slowly administered intravenously). Following surgery, 600,000 units of benzathine pencillin-G mixed with 300,000 units of potassium pencillin-G (all Purpose Bicillin Injection, Wyeth Laboratories) were administered intramuscularly as a prophylactic measure against infection resulting from the surgical procedure. For 72 hr following surgery, each lever-press response exceeding 100 g of force and occurring during illumination of the stimulus light resulted in the intravenous administration of 0.2 cc/kg of sterile physiological saline. During this three day period the stimulus light was constantly illuminated except during an injection cycle. All lever-press responses were recorded on an Esterline Angus event recorder. Programmed injections were administered every 4 hr in an effort to retard clot formation in the catheter. During this saline period, none of the animals emitted more than twelve lever-press responses within any 24 hr segment.

Following this 72 hr period the saline solution was replaced by a solution of cocaine hydrochloride in sterile physiological saline. The stimulus light was illuminated 24 hr a day for five days (except during an injection cycle). Each lever-press occurring while the light was illuminated resulted in the administration of 0.2 mg/kg of cocaine hydrochloride. Solutions of cocaine hydrochloride were prepared every fifth day. As previously mentioned, programmed injections occurred every 4 hr. The duration of an injection cycle lasted from 35 -50 sec depending on the animals body weight. Each animal initiated self-administration behavior well within this five day period. Drug access was then reduced to a daily (7 days a week) 4-hr session (10:00 a.m. - 2:00 p.m.). As previously indicated, drug availability was indicated by the illumination of the stimulus light. Lever-presses in the absence of the illuminated stimulus light, were recorded but had no consequence. Within two weeks daily drug intake was stable (< 10% change in daily drug intake over five consecutive sessions) for each animal. Furthermore, stimulus control of responding in each animal was evidenced by the small number of responses (< 10) emitted by any subject during the twenty hour period separating successive sessions. Programmed injections continued to be administered every 4 hr. If a catheter became inoperative during the course of the study it was either replaced or a catheter implanted in the opposite internal jugular vein. If catherization was required a subsequent seven day reconditioning period was interposed to regain behavioral stability.

The effects of acute pretreatment with atropine, methylatropine, and physostigmine on stable cocaine self-administration behavior were ascertained in each subject. Four dosages of physostigmine (0.05–0.5 mg/kg), four dosages of methylatropine ((0.5 - 3.0 mg/kg) and six dosages of atropine (0.125 3.0 mg/kg) were studied. All dosages were calculated on the basis of the salt form. All pretreatment solutions were prepared using a sterile physiological saline vehicle on the day on which they were administered. The solutions were refrigerated until 30 min prior to initiating testing with the next drug. The sequence of dosage testing with each drug in each animal was randomized. The pretreatment drug was administered intramuscularly 5 min prior to session onset. The pretreatment drug volume was kept constant at 0.5 cc with the concentration of the pretreatment solution being varied. Three sessions separated drug pretreatment sessions in an attempt to insure no carry over of drug effects from the previous pretreatment test session. Sessions in which 0.5 cc of sterile physiological saline were administered intramuscularly 5 min prior to session onset were interspersed between drug pretreatment sessions (after the frequency of self-administration behavior had returned to baseline rates) to control for conditioning of the pretreatment procedure. The percentage change in cocaine self-administration seen in drug pretreatment sessions was compared to that which occurred in saline pretreatment sessions.

RESULTS

The effects of atropine pretreatment on cocaine self-administration are illustrated in Fig. 1. Pretreatment with 0.125 mg/kg produced little change in the rate of this behavior in any of the subjects; however, a dose of 0.25 mg/kg increased this behavior above control values in three of the four animals. The mean increase was 21%, however, the effects of this dosage were not statistically significant when compared to the effects of saline treatment. As the pretreatment dosage of atropine was increased from 0.25 to 2.0 mg/kg a dose-related increase in cocaine self-administration occurred. The differences in the mean increase in cocaine self-administration produced by these three dosages were not statistically significant ($p>0.05$ via Student's t-test) from each other. The mean increase in this behavior seen with the 0.5 mg/kg dose was 42%; the 1.0 mg/kg dose was 58%; and the 2.0 mg/kg dose was 70%. The effect produced by each of these three dosages was significantly different ($p<0.05$) from saline treatment. Three of the four animals, following treatment with 0.5 mg/kg of atropine, exhibited an increase in this behavior above control values; whereas, all four of the animals self-administered more cocaine following treatment with 1.0 and 2.0 mg/kg of atropine.

Figure 2 demonstrates that the latency of atropine's effect on cocaine self-administration decreased as the dosage of atropine was increased from 0.25 to 2.0 mg/kg. The 0.5 mg/kg dose of atropine increased the frequency of this behavior in the last three hours of the session; whereas, the 1.0 and 2.0 mg/kg dosages potentiated the behavior primarily during the first two hours of the session. By the fourth hour of the session, the effects of the 1.0 and 2.0 mg/kg dosages of atropine had dissipated. Pretreatment with 3.0 mg/kg of atropine produced a mean increase in cocaine self-administration of 25% which was not statistically different from that produced by either 0.125 or 0.25 mg/kg of atropine or by saline. In two of the four animals,

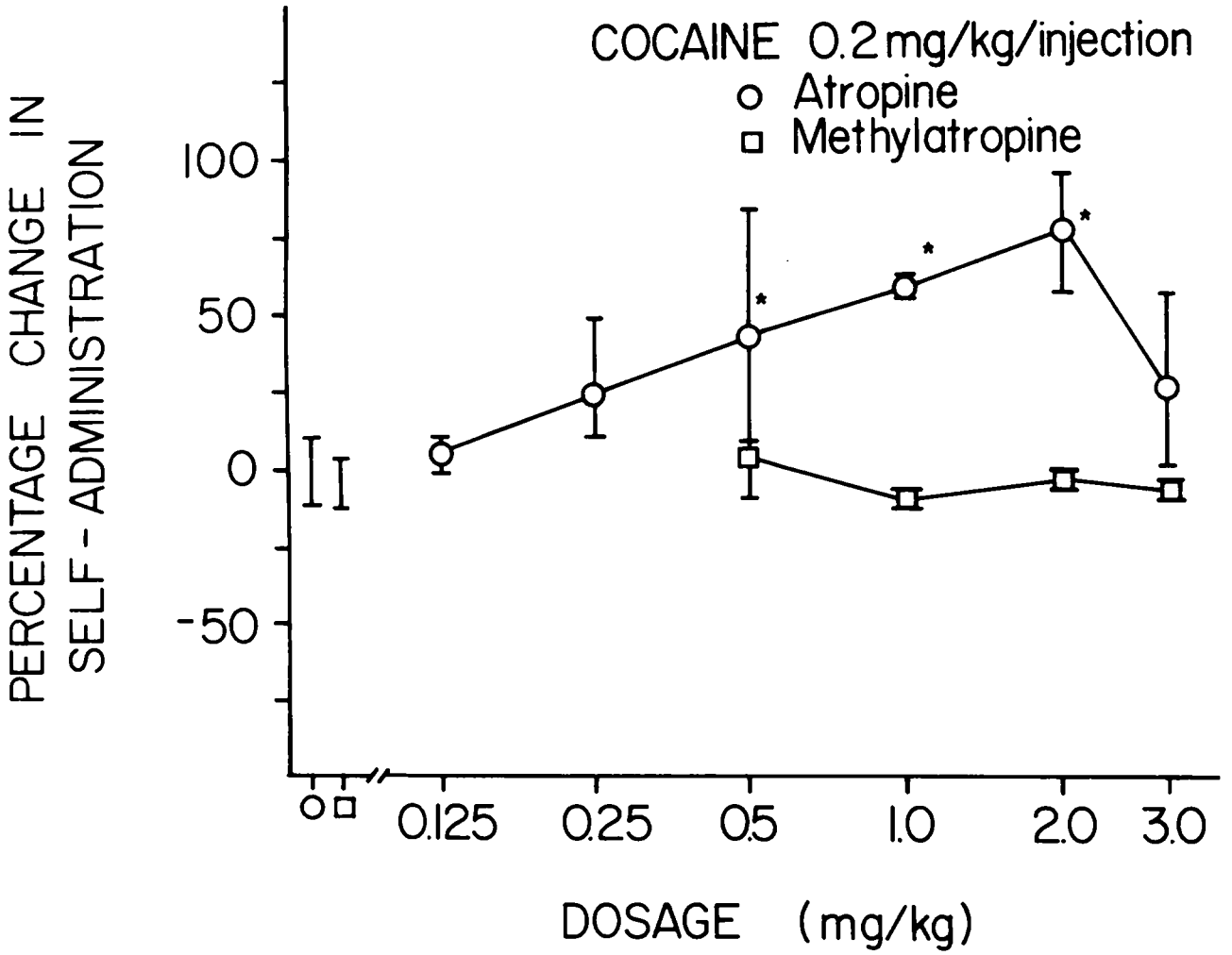

FIG. 1. Mean percentage change in cocaine self-administration as a function of the pretreatment dosage of atropine (○—○) and methylatropine (□—□). Each point represents the mean of four animals and the vertical lines illustrate the range of change within these animals. The brackets at the left represent the range of change in cocaine self-administration which occurred following pretreatment with saline. Five of these control sessions were conducted in each animal. An asterisk (*) denotes those points significantly different from saline ($p<0.05$).

this dosage of atropine did increase this behavior above control values but not to the extent seen with the 2.0 mg/kg dosage. In those animals in which the 3.0 mg/kg dosage stimulated cocaine self-administration, the increase occurred during the initial half of the session.

Figure 1 also illustrates the effects of methylatropine treatment on this behavior. Methylatropine in dosages of 0.5, 1.0, 2.0, and 3.0 mg/kg did not change cocaine self-administration during any part of any session in any of the four subjects.

The effects of physostigmine on intravenous cocaine self-administration are represented in Fig. 3. Pretreatment with 0.05 mg/kg of physostigmine did not significantly alter this beahvior in any of the four animals. However, treatment with 0.1, 0.25 and 0.5 significantly decreased cocaine self-administration behavior when compared to saline ($p<0.05$). This behavior was depressed below control values by these dosages in all four animals. The mean percentage decrease in this behavior produced by 0.1 mg/kg of physostigmine was 59%; by 0.25 mg/kg, 69%; and by 0.5 mg/kg, 92%. Vomiting, diarrhea and malaise occurred during these sessions in which cocaine self-administration behavior was suppressed and the severity of these effects appeared to be dose-related. The time course of the effects of physostigmine on cocaine self-administration in one subject is illustrated in Fig. 4. These data are essentially identical to those seen with the other three animals. Pretreatment with 0.05 mg/kg produced no change in cocaine self-administration during any period of the session. Cocaine intake was also not altered during the first hour of the session following pretreatment with either 0.1 or 0.25 mg/kg. However, decreases in cocaine self-administration were produced during the latter three hours of the session with these dosages. Pretreatment with 0.5 mg/kg almost completely suppressed this behavior during the entire session.

DISCUSSION

Atropine sulfate has been shown to block the neurotransmitter function of acetylcholine at the parasym-

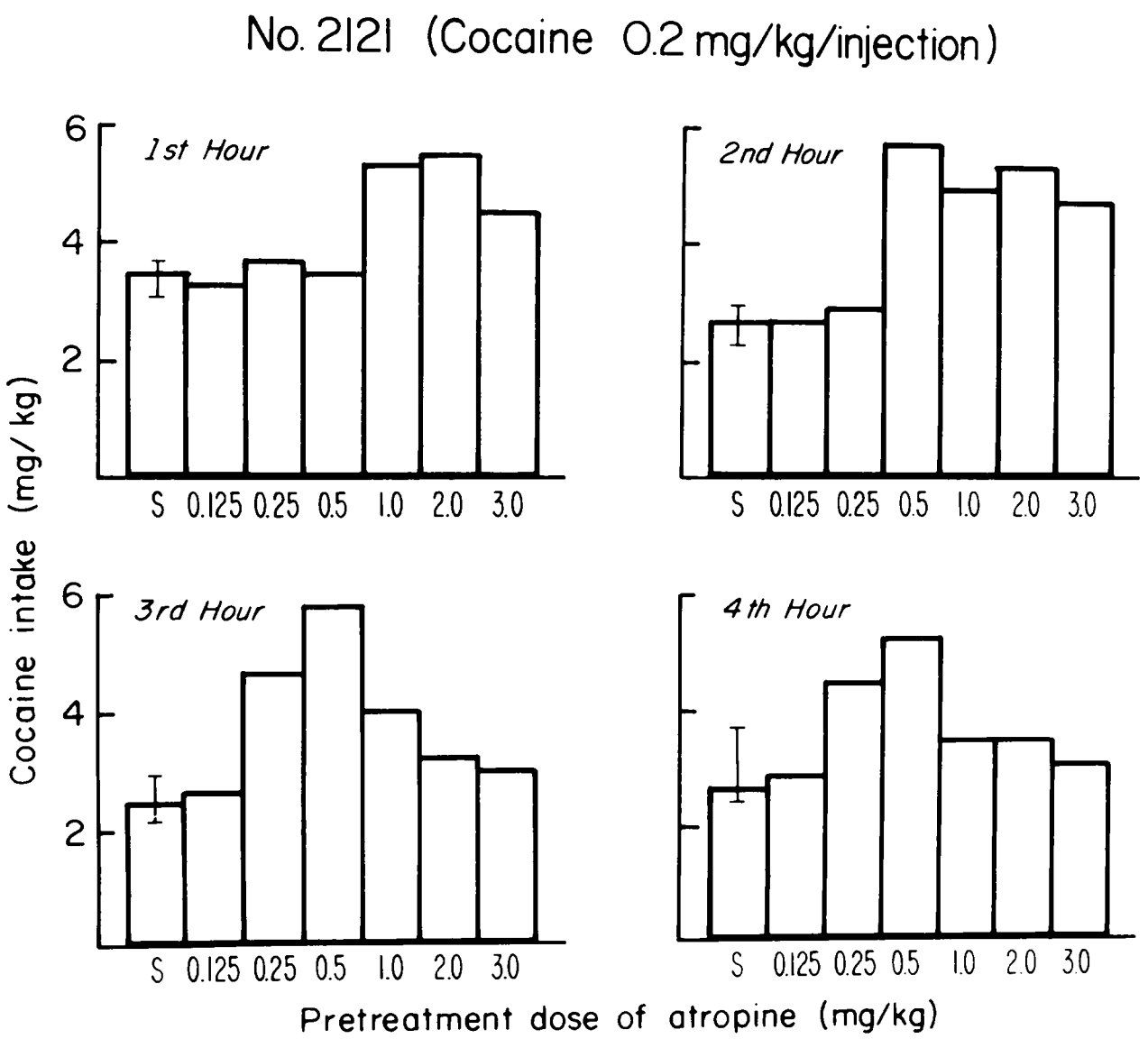

FIG. 2. Alteration in hourly cocaine intake in a typical animal as a function of the pretreatment dosage of atropine. The height of the bars illustrates the amount of cocaine self-administration during that hour following pretreatment with a given dose of atropine. The height of vertical bars over the letter S represents mean cocaine intake in that period, following pretreament with saline on five different occasions. The vertical lines represent the range of cocaine intake for these five control sessions.

pathetic neuroeffector junction and also to have a definite central action [2]. Methylatropine has a similar peripheral action [10] but does not exert central atropine-like actions as readily as atropine i.e. in equimolar doses [15,16]. Therefore, by using these two agents it was possible to compare the effects of central and peripheral cholinergic blockage on cocaine self-administration behavior. The increase in cocaine self-administration behavior seen following atropine pretreatment, but not following methylatropine pretreatment suggests that this effect is not due to peripheral cholinergic blockage and secondly that this effect could possibly be due to central cholinergic blockage. Even if it is assumed that the latter action is responsible for this behavioral facilitation, the result of this biochemical interaction which leads to the increase in cocaine intake is uncertain. A similar increase in the frequency of cocaine self-administration occurs following a reduction in the unit dosage of cocaine [21] and also following pretreatment with chlorpromazine [22,23] and also following treatment with alpha methylparatyrosine (manuscript in preparation). Furthermore, it has been demonstrated in the rat that treatment with alpha methylparatyrosine reduces primary and secondary reinforcement associated with d-amphetamine self-administration [8]. Therefore, perhaps atropine is antagonizing the reinforcing efficacy of cocaine which may be analogous to reducing the unit dosage.

However, this explanation does not seem plausible when one considers the interactions reported between atropine

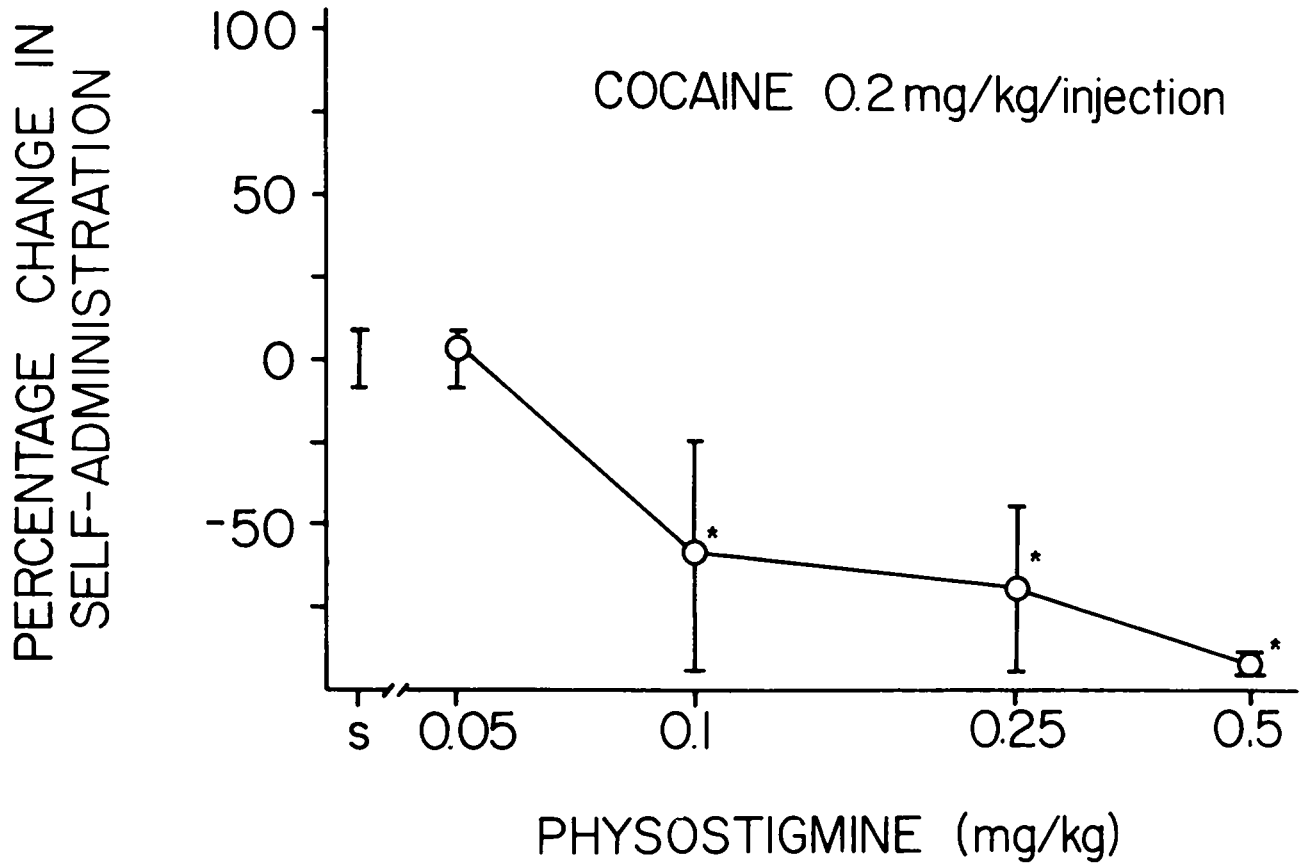

FIG. 3. Mean percentage change in cocaine self-administration as a function of the pretreatment dosage of physostigmine. Each point represents the mean of four animals and vertical lines illustrate the range of change within these animals. The brackets at the left represent the range of change in cocaine self-administration which occurred following pretreatment with saline. Five of these control sessions were conducted in each animal. An asterisk (*) denotes those points significantly different from saline ($p<0.05$).

and psychomotor stimulants on other behaviors. Atropine has been shown to enhance the stimulant action of both cocaine and d-amphetamine on continuous avoidance responding [6,18]. Furthermore, atropine has been demonstrated to facilitate hypermotility produced by cocaine and d-amphetamine [11]. The ability of central cholinergic blocking agents to enhance the toxicity of amphetamine in grouped mice has also been reported [14]. The ability of atropine to enhance the facilitory effects of methamphetamine on intracranial self-stimulation has also been shown [19]. Furthermore, it has been demonstrated that amphetamine-induced stereotypy and hyperactivity in rats were enhanced by anticholinergic drugs and antagonized by cholinergic agents [1]. Therefore, since in all of these reports, atropine enhanced the actions of psychomotor stimulants, one would have expected to see a decrease in the frequency of cocaine self-administration since increases in unit dosage result in a decrease in cocaine self-administration behavior [21]. Pretreatment with other agents which facilitate many of the behavioral and autonomic actions of cocaine e.g. d-amphetamine, phenmetrazine and imipramine, decrease the frequency of cocaine self-administration behavior [23].

The present investigators have postulated that either an aversive or a nonspecific behavioral disrupting action of the psychomotor stimulants (e.g. prominent stereotypy) may function in limiting the amount of these agents which is self-administered daily, and therefore provides for a stable self-administration behavioral baseline[21]. Potentiation of these effects by atropine would tend to reduce the frequency of this behavior. However, if these aversive or behavioral disrupting actions are cholinergic in nature then atropine would antagonize them. This antagonism of an aversive effect of cocaine would result in an increase in the reinforcing efficacy of the cocaine, which is reflected as an increase in cocaine self-administration behavior.

A second possible explanation for the effect of atropine on this behavior is suggested in a review of the effects of this agent on behavior [7]. On the basis of his literature review Carlton hypothesized that a cholinergic inhibitory system functions in antagonizing behavior activations. Atropine would then block such an inhibitory system thereby permitting behavioral activation which in the case of the present data would result in an increase in cocaine self-administration. Therefore, perhaps there is no atropine-cocaine interaction per se and that the increase in cocaine self-administration is due to a non-specific behavioral disinhibition produced by atropine.

The results of pretreatment with physostigmine are very difficult to interpret since decreases in cocaine self-adminis-

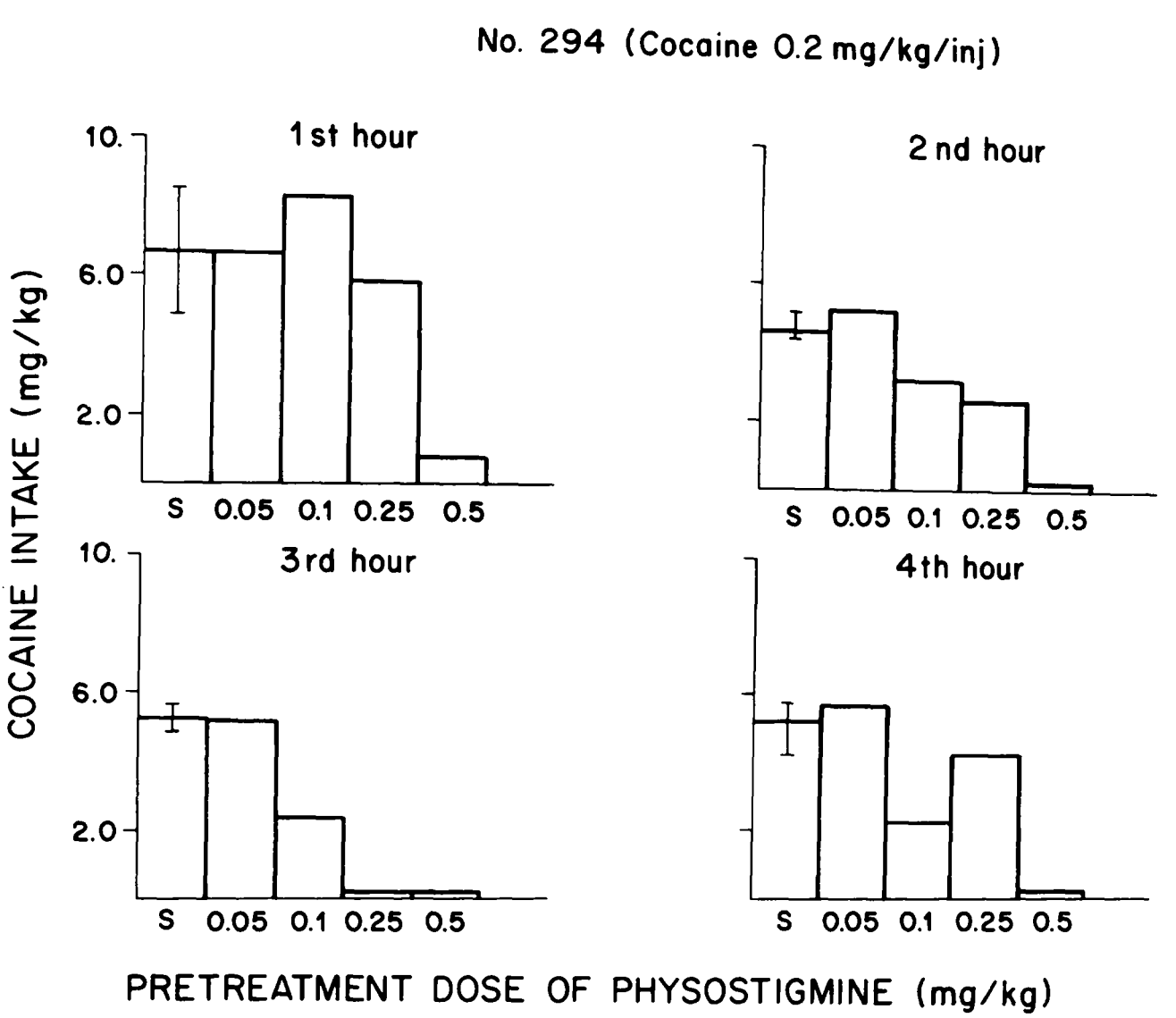

FIG. 4. Alteration in hourly cocaine intake in a typical animal as a function of the pretreatment dosage of physostigmine. The height of the bars illustrates the amount of cocaine self-administration during that hour following pretreatment with a given dosage of physostigmine. The height of the vertical bars over the letter S represents mean cocaine intake in that period following pretreatment with saline on five different occasions. The vertical lines represent the range of cocaine intake for these five control sessions.

tration were only seen with pretreatment dosages which produced vomiting, diarrhea and malaise. Conceivably the decrement in cocaine self-administration was the result of this pathological state. However, treatment with dosages of reserpine which produce a similar degree of diarrhea, malaise and behavioral depression does not suppress cocaine self-administration (manuscript in preparation). Therefore, perhaps the depression in cocaine self-administration following physostigmine pretreatment was unrelated to the malaise and nausea.

Physostigmine in general tends to depress conditioned behavior. It decreases active [17] and passive avoidance responding [3] as well as food reinforced responding [4]. These effects on both avoidance responding and food-reinforced responding have been confirmed by other investigators [20]. This response suppression can be antagonized with atropine but not with amphetamine. Physostigmine which is a central and peripheral acting anticholinesterase agent [13] facilitates cholinergic transmission and would activate the cholinergic inhibitory system previously postulated and thereby would decrease ongoing behavior.

One must also consider the possibility that physostigmine may be enhancing aversive or behavioral disruptive actions of cocaine, if these are functioning in controlling this behavior and are mediated by cholinergic systems. Presently there is little evidence to support this concept of facilitation of the behavioral effects of the psychomotor stimulants by anticholinesterase agents. However, the peripherally induced salivation seen with cocaine would certainly be enhanced by physostigmine. Certainly many peripheral autonomic actions of cocaine based on blocking the reuptake of the adrenergic transmitter, norepinephrine,

would tend to be reduced by the concomitant use of low dosages of a cholinesterase inhibitor. However, larger doses may via sympathetic ganglionic stimulation and adrenal medullary stimulation potentiate the autonomic effects of cocaine. If these autonomic effects are aversive to the animal then perhaps it is via this mechanism that physostigmine reduces cocaine self-administration.

It is also quite conceivable that the animals experiencing the vomiting and diarrhea attribute these effects to the cocaine if there is a temporal congruity between the effect and emission of the lever-press operant. This in turn may be analogous to punished responding and therefore the animals refrain from additional self-administration behavior.

Although these data suggest that the central cholinergic system may mediate cocaine reinforcement, it presently is more parsimonious to consider the effects of these agents on this behavior to be due to an interaction with the factors which limit or regulate cocaine self-administration.

REFERENCES

1. Arnfred, T. and A. Randrup. Cholinergic mechanism in brain inhibiting amphetamine stereotyped behavior. *Acta pharmac. tox.* **26:** 384–394, 1968.
2. Bradley, P. and J. Elkes. The effect of atropine, hyoscyamine, physostigmine and neostigmine on the electrical activity of the brain of the conscious cat. *J. Physiol.* **120:** 14P–22P, 1953.
3. Bures, J., Z. Bohdanecky and T. Weiss. Physostigmine induced hippocampal theta activity and learning in rats. *Psychopharmacologia (Berl.)* **3:** 254–263, 1962.
4. Cardo, D. Action de l'amphetamine dextrogyre et de l'eserine sur un conditionnement de fuite et sur les phenomenes de discrimination. *J. Physiol. (Paris)* **5:** 845–853, 1959.
5. Carlton, P. Augmentation of the behavioral effects of amphetamine by scopolamine. *Psychopharmacologia (Berl.)* **2:** 377–380, 1961.
6. Carlton, P. and P. Didamo. Augmentation of the behavioral effects of amphetamine by atropine. *J. Pharmac. exp. Ther.* **132:** 91–96, 1961.
7. Carlton, P. L. Cholinergic mechansims in the control of behavior by the brain. *Psychol. Rev.* **70:** 19–64, 1963.
8. Davis, W. M. and S. G. Smith. Blocking effect of alpha-methyltyrosine on amphetamine based reinforcement. *J. Pharm. Pharmac.* **25:** 174–177, 1973.
9. Deneau, G., T. Yanagita and M. H. Seevers. Self-administration of psychoactive substances by the monkey. *Psychopharmacologia (Berl.)* **16:** 30–48, 1969.
10. Fink, L. and P. Cervoni. Ganglionic blocking actions of atropine and methylatropine. *J. Pharmac. exp. Ther.* **109:** 372–376, 1953.
11. Galambos, E., A. Klara Pfeifer, L. Gyorgy and J. Molnar. Study on the excitation induced by amphetamine, cocaine and alpha-methyltryptamine. *Psychopharmacologia (Berl.)* **11:** 122–129, 1967.
12. Goldberg, S., F. Hoffmeister, U. Schlichting and W. Wuttke. A comparison of pentobarbital and cocaine self-administration in rhesus monkeys: effects of dose and fixed-ratio parameter. *J. Pharmac. exp. Ther.* **179:** 277–283, 1971.
13. Koelle, G. Anticholinesterase agents. In: *The Pharmacological Basis of Therapeutics,* 4th Edition, edited by L. Goodman and A. Gilman. New York: MacMillan Company, 1970, 442 p.
14. Mennear, J. Interactions between central cholinergic agents and amphetamine in mice. *Psychopharmacologia (Berl.)* **7:** 107–114, 1965.
15. Paul-David, J., J. L. Riehl and K. R. Unna. Quantification of effects of depressant drugs on EEG activation response. *J. Pharmac. exp. Ther.* **129:** 69–74, 1960.
16. Pfeiffer, C. C. and E. H. Jenney. The inhibition of the conditioned response of the counteraction of schizophrenia by muscarinic stimulation of the brain. *Ann. N. Y. Acad. Sci.* **66:** 753-764, 1957.
17. Rosecrans, J., A. Dren and E. F. Domino. Effects of Physostigmine on rat brain acetylcholine, acetylcholinesterase and avoidance behavior. *Fedn Proc.* **25:** no. 2, 261, 1966.
18. Scheckel, C. and E. Boff. Behavioral effects of interacting imipramine and other drugs with d-amphetamine, cocaine and tetrabenazine. *Psychopharmacologia (Berl.)* **5:** 198–206, 1964.
19. Stein, L. New methods for evaluating stimulants and antidepressants. In: *The First Hahnemann Symposium on Psychosomatic Medicine,* edited by J. Nodine and J. Moyer. Philadelphia, Pennsylvania: Lea and Febiger Inc., 1962, 297 p.
20. Vaillant, G. A comparison of antagonists of physostigmine-induced suppression of behavior. *J. Pharmac. exp. Ther.* **157:** 636–648, 1967.
21. Wilson, M. C., M. Hitomi and C. R. Schuster. Psychomotor stimulant self-administration as a function of dosage per injection in the rhesus monkey. *Psychopharmacologia (Berl.)* **22:** 271–281, 1971.
22. Wilson, M. C. and C. R. Schuster. The effects of chlorpromazine on psychomotor stimulant self-administration in the rhesus monkey. *Psychopharmacologia (Berl.)* **26:** 115–126, 1972.
23. Wilson, M. C. and C. R. Schuster. The effects of stimulants and depressants on cocaine self-administration behavior in the rhesus monkey. *Psychopharmacologia (Berl.)* **31:** 291–304, 1973.
24. Woods, J., and C. R. Schuster. Reinforcement properites of morphine cocaine, and SPA as a function of unit dose. *Int. J. Addict.* **3:** 231–240, 1968.