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Cholinergic Modulation of Stimulant-Induced Behavior[1]

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GONZALEZ, L. P. AND E. H. ELLINWOOD, JR. *Cholinergic modulation of stimulant-induced behavior.* PHARMACOL BIOCHEM BEHAV 20(3) 397–403, 1984.—Stimulant-induced stereotypy, presumably mediated by nigrostriatal dopaminergic neurons, can be altered by the administration of cholinergic agonists or antagonists. Cholinergic, striatal interneurons have been postulated as the site of these effects, although the specific site of interaction between cholinergic and dopaminergic systems is unknown. The study reported here examined the effects of the cholinesterase inhibitor physostigmine, and several other cholinergic and anticholinergic drugs, on stimulant-induced behavior. Stereotypy was inhibited by physostigmine to the same degree whether induced by direct (apomorphine) or by indirect acting (amphetamine and methylphenidate) stimulants. The results are interpreted as indicating that the site of cholinergic modulation of stimulant-induced stereotypy is postsynaptic to the dopaminergic neurons which mediate stereotypy.

Stimulants Amphetamine Methylphenidate Apomorphine Physostigmine Stereotypy

THE research of many investigators over the past few years has supported the hypothesis that the patterned stereotypies induced in animals by high doses of amphetamine or amphetamine-like drugs are dependent to a large extent upon a dopaminergic nigrostriatal projection in the central nervous system [9, 11, 24]. Biochemical [34,38], pharmacological [2, 34, 39], and electrophysiological [52] studies suggest that this dopaminergic system interacts with cholinergic neuronal elements within the striatum. Furthermore, cholinergic systems are implicated as modulators of the primary dopaminergic effects on behavior since stimulant-induced stereotypy is potentiated by anticholinergic drugs and inhibited by cholinergic agonists [3, 32, 37, 45, 47].

Although the specific neuroanatomical relationship between dopaminergic and cholinergic elements is currently unknown [13], the cholinergic interneurons of the striatum have been suggested to function in a feedback regulation of dopaminergic nigrostriatal projections [25]. These interneurons are also believed to directly modulate activity in a major output pathway from the striatum through which this structure influences motor behavior [54]. In addition, some investigators have presented evidence that cholinergic receptors are located on nigral dopaminergic neurons and may modulate dopaminergic activity [13, 30, 38, 43].

The stimulants amphetamine, methylphenidate, and apomorphine each produce similar changes in the behavior of rodents. While all three are believed to act through stimulation of dopamine receptors, their specific mechanisms of action differ. Amphetamine is believed to act indirectly by promoting the release of newly synthesized dopamine [5, 8, 41, 48] and its effects on stereotypy can be blocked by blocking dopamine synthesis with alpha-methylparatryosine [46,51]. Methylphenidate also enhances dopamine release [21], but its behavioral effects can be blocked by reserpine but not by alpha-methylparatyrosine, suggesting a dependence upon a presynaptic vescicular pool of dopamine [44]. Apomorphine, on the other hand, is a direct dopamine receptor stimulant, which can induce stereotypy in the absence of presynaptic dopamine [18, 19, 20, 26].

If cholinergic systems function postsynaptically to dopaminergic neurons in the modulation of the motor outflow which mediates stimulant-induced behavior, all three stimulants should be equally susceptible to disruption by cholinergic drugs. Effects on cholinergic neurons presynaptic to the functionally relevant dopaminergic neurons, however, would be most likely to alter the response to the indirect acting stimulants, amphetamine and methylphenidate, as would effects on a nigrostriatal feedback loop since this feedback loop presumably regulates dopamine synthesis and release [6, 7, 10].

The experiments presented here were performed to determine the involvement of cholinergic mechanisms in

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stimulant-induced stereotypy and, by comparing direct and indirect acting stimulants, to determine whether the observed effects of cholinergic drugs could be the result of presynaptic modulation of dopamine neurons, or similarly alterations in the feedback regulation of the nigrostriatal pathway, or alternately if these effects could be due to a postsynaptic effect on a motor output pathway.

METHOD

Subjects

The subjects for these experiments were 210 male, Sprague-Dawley rats, 60 to 90 days old, and weighing 200 to 250 g. Animals were housed in individual cages with free access to food and water, and were maintained for at least seven days under the same conditions of environment, diet, and daily handling before any experimental treatment.

Apparatus

The measurement of stereotypy was performed in a Stoelting activity monitor, modified to permit quantification of restricted, repetitive behaviors. This apparatus has been described in detail elsewhere [17]. Briefly, the motility monitor consists of a pair of parallel plates connected to a Stoelting movement sensoring module. An animal is placed in the center of a capacitance field generated between the plates such that the movement of the animal disrupts the field. Based upon this disruption, the motility monitor produces an analog signal with a frequency of oscillation equal to the frequency of occurrence of movements within the field of the monitor. Quantification of movement is accomplished by spectral analysis of the frequency components of the analog output of the motility monitor. The resulting amplitude-frequency distribution has been shown [16,17] to accurately depict the occurrence of specific repetitive movements (sniffing, licking, head bobbing, etc.). The power spectrum of the transduced signal provides a statistically significant dose-response curve for amphetamine [15, 16, 23] and thus permits the quantification of stimulant-induced stereotypy.

For the measurement of motility, animals were placed in Plexiglas chambers, 19.0×13.0×8.0 cm, which were then positioned within the movement sensor of the motility monitor. Analog-to-digital conversion of the transduced signal and subsequent analyses were performed on a DEC PDP mini-computer.

Drugs

The drugs used in these studies included apomorphine HCl (Merck), d-amphetamine sulphate (Sigma), methylphenidate HCl (Ciba), physostigmine salicylate (Sigma), neostigmine methylsulphate (Sigma), scopolamine HBr (Sigma), and methylscopolamine Br (Upjohn). All drugs were prepared on the day of an experiment. Drugs were dissolved in 0.9% saline in a volume of 1.0 ml/kg body weight, and were administered intraperitoneally. Drug doses are expressed in terms of the salt.

Procedures

Subjects were randomly divided into one of the treatment groups listed in Table 1. Following a 15 min period for adaptation to the apparatus, motility was monitored for a period of 40 seconds. A drug pretreatment was then administered, as appropriate to the group designation of a subject. Motility was again monitored for 40-second periods, ten and 20 minutes after drug pretreatment. Immediately after the latter period, a second drug treatment was administered. Motility was monitored as before, immediately after this second drug injection and again at five, ten, 20, 30, and 40 min after this injection.

A Fast Fourier Transform was used to obtain power spectra for each one-second segment of motility data, and the spectra were averaged across the 40 seconds of each sampling period. Following a log transformation of the mean power spectra, an analysis of variance with repeated measures was used to determine the significance of group differences at the various sampling periods, with Duncan's multiple range test used for individual post-hoc group comparisons.

TABLE 1
EXPERIMENTAL TREATMENT GROUPS

Pretreatment	Final Drug Treatment	N
Saline	Saline	24
Physostigmine,		
0.15 mg/kg	Saline	6
0.30 mg/kg	Saline	12
0.60 mg/kg	Saline	18
Scopolamine,		
0.40 mg/kg	Saline	6
1.00 mg/kg	Saline	6
Methylscopolamine,		
1.00 mg/kg	Saline	6
Neostigmine,		
0.44 mg/kg	Saline	6
Saline	d-Amphetamine, 3.0 mg/kg	6
Physostigmine,		
0.15 mg/kg	d-Amphetamine, 3.0 mg/kg	6
0.30 mg/kg	d-Amphetamine, 3.0 mg/kg	6
0.60 mg/kg	d-Amphetamine, 3.0 mg/kg	6
Saline	d-Amphetamine, 6.0 mg/kg	6
Physostigmine,		
0.15 mg/kg	d-Amphetamine, 6.0 mg/kg	6
0.30 mg/kg	d-Amphetamine, 6.0 mg/kg	6
0.60 mg/kg	d-Amphetamine, 6.0 mg/kg	6
Saline	Methylphenidate, 6.0 mg/kg	12
Physostigmine,		
0.30 mg/kg	Methylphenidate, 6.0 mg/kg	6
0.60 mg/kg	Methylphenidate, 6.0 mg/kg	6
Scopolamine,		
0.40 mg/kg	Methylphenidate, 6.0 mg/kg	6
1.00 mg/kg	Methylphenidate, 6.0 mg/kg	6
Methylscopolamine,		
1.00 mg/kg	Methylphenidate, 6.0 mg/kg	6
Neostigmine,		
0.44 mg/kg	Methylphenidate, 6.0 mg/kg	6
Saline	Methylphenidate, 12.0 mg/kg	6
Physostigmine,		
0.30 mg/kg	Methylphenidate, 12.0 mg/kg	6
0.60 mg/kg	Methylphenidate, 12.0 mg/kg	6
Saline	Apomorphine, 0.5 mg/kg	6
Physostigmine,		
0.60 mg/kg	Apomorphine, 0.5 mg/kg	6

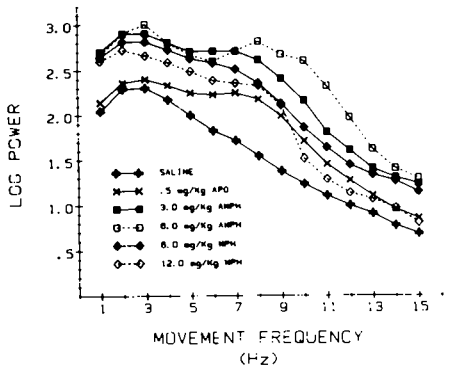

FIG. 1. Stimulant-induced motility following saline pretreatment. The mean power spectra are shown for subjects receiving a saline pretreatment with a subsequent injection 20 minutes later of either saline, amphetamine (AMPH), methylphenidate (MPH), or apomorphine (APO). Spectra were computed from data obtained 10 minutes after this second injection. In this figure, arbitrary log power units are represented on the Y-axis and movement frequency on the X-axis.

RESULTS

Stimulant-Induced Motility Following Saline Pretreatment

Consistent with our previous report [23], amphetamine (3.0 and 6.0 mg/kg) administered to saline pretreated animals produced a general increase in power at all observed frequencies (1 to 15 Hz) with the greatest change occurring in a band of movement frequencies centered at 8 Hz (Fig. 1). Power in this frequency band has been shown [17] to correspond to the occurrence of restricted, stereotyped sniffing behavior. Amphetamine produced significant ($p<0.001$) changes in the motility of amphetamine-treated animals compared before and after drug administration; differences post-injection were also significant ($p<0.001$) for amphetamine-treated versus saline-treated animals. These changes in motility were significant throughout the 40-minute post-amphetamine observation period.

Methylphenidate (6.0 and 12.0 mg/kg) produced effects in saline pretreated animals which were similar to the effects of amphetamine described above (Fig. 1). This included an increase in power at all frequencies with a prominent increase in 8 Hz movements throughout the post-drug observation period ($p<0.001$).

In saline pretreated animals, apomorphine (0.5 mg/kg) also induced a significant ($p<0.001$) increase in motility, but this effect was limited to the movement band centered at 8 Hz (Fig. 1). Apomorphine also differed from the other stimulants examined in this study in that its duration of action was much shorter, with motility returning to control values by 30 minutes after injection.

The effects of all three stimulants examined were most evident in the 8 Hz movement band; thus, the analyses which follow were based upon statistical comparisons of differences in 8 Hz motility.

Effects of Pretreatments Alone on Motility

None of the drug pretreatments used in this study (Table 1) significantly ($p>0.05$) altered the motility spectra of animals, either during the period prior to stimulant administration or when followed by the administration of saline (Fig. 2).

Physostigmine Effects on Stimulant-Induced Motility

Amphetamine. Physostigmine significantly altered amphetamine-induced motility in a dose-related manner (Fig. 3). The increased 8 Hz movements produced by amphetamine (3.0 and 6.0 mg/kg) were significantly ($p<0.002$) reduced in animals pretreated with 0.6 mg/kg physostigmine when compared to amphetamine-injected animals pretreated with saline. These effects, although decreasing with time, were significant for the entire 40 min observation period for animals receiving 3.0 mg/kg amphetamine, but were only significant during the first 10 min following the administration of 6.0 mg/kg amphetamine. Pretreatment with 0.3 mg/kg physostigmine also reduced the effects of 3.0 mg/kg amphetamine ($p<0.001$), but did not alter the effects of 6.0 mg/kg amphetamine ($p>0.05$). This time-dependent effect was significant, in animals receiving the low dose of amphetamine, for the entire 40 min observation period. The lowest dose of physostigmine (0.15 mg/kg) did not significantly ($p>0.05$) alter the effects of either 3.0 or 6.0 mg/kg amphetamine. Although the higher doses of physostigmine

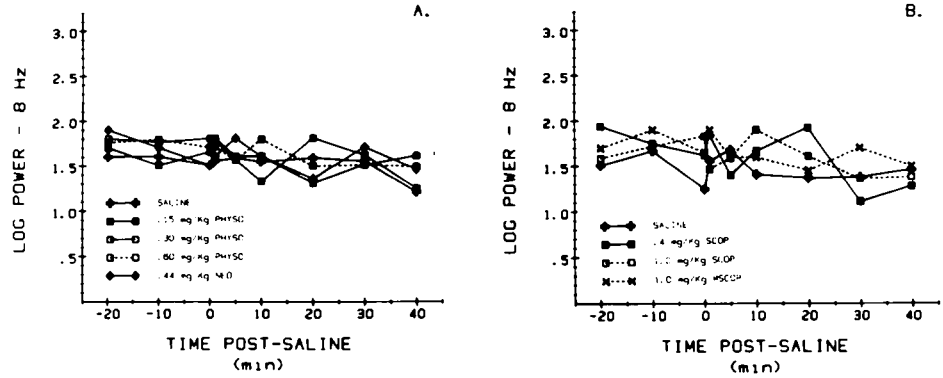

FIG. 2. Effects of pretreatment drugs on 8 Hz motility before and after saline treatment. The mean log power of 8 Hz movements is presented along the Y-axis for groups of rats receiving pretreatments which consisted of either (A) the cholinergic agonists physostigmine (PHYSO) or neostigmine (NEO) or (B) the antagonists scopolamine (SCOP) or methylscopolamine (MSCOP). Time before and after saline treatment is presented on the X-axis (injection at time zero). Pretreatments were administered just after the −20 minute period.

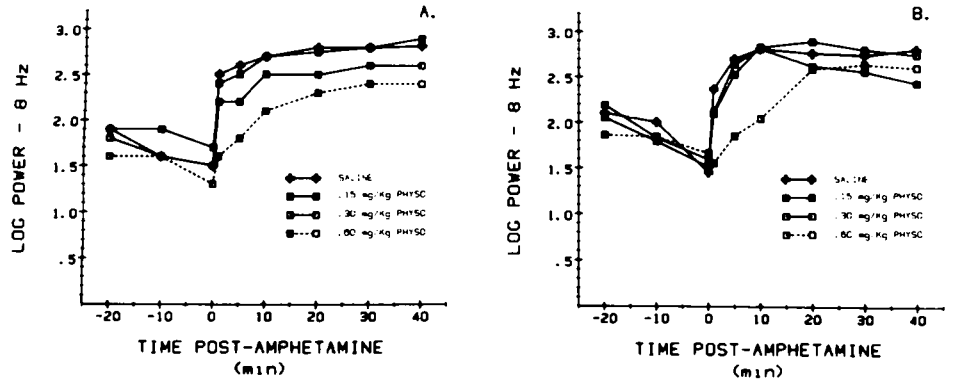

FIG. 3. Physostigmine inhibition of amphetamine-induced 8 Hz motility. The mean log power of 8 Hz movements is shown for animals receiving pretreatments of saline or physostigmine (PHYSO), followed by either (A) 3.0 mg/kg d-amphetamine or (B) 6.0 mg/kg d-amphetamine. Time before and after amphetamine treatment is presented on the X-axis (injection at time zero). Pretreatments were administered just after the −20 minute period.

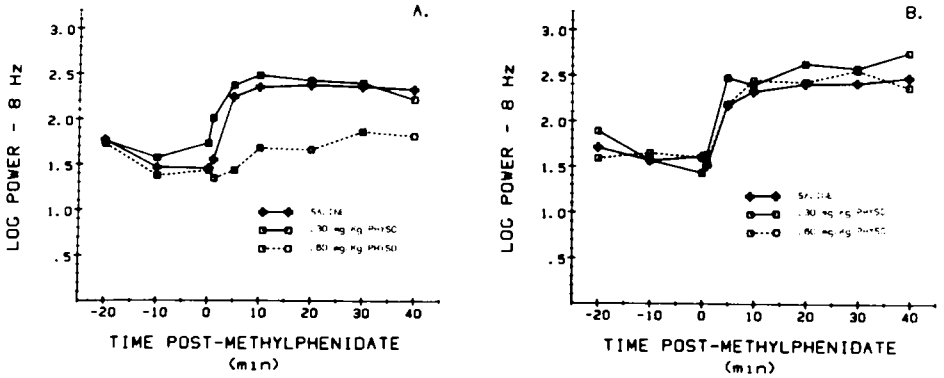

FIG. 4. Physostigmine inhibition of methylphenidate-induced 8 Hz motility. The mean log power of 8 Hz movements is shown for animals receiving pretreatments of saline or physostigmine (PHYSO), followed by either (A) 6.0 mg/kg methylphenidate or (B) 12.0 mg/kg methylphenidate. Time before and after methylphenidate treatment is presented on the X-axis (injection at time zero). Pretreatments were administered just after the −20 minute period.

significantly reduced amphetamine-induced motility, all of the physostigmine-treated groups which received amphetamine (3.0 or 6.0 mg/kg) exhibited significantly more 8 Hz motility than did saline animals receiving similar pretreatment ($p<0.001$).

Methylphenidate. The effects of 6.0 mg/kg methylphenidate were significantly reduced ($p<0.001$) by pretreatment with 0.6 mg/kg physostigmine (Fig. 4). This effect was significant for the entire observation period. Subjects pretreated with this dose of physostigmine which also received 6.0 mg/kg methylphenidate did not differ significantly ($p>0.05$) from similarly pretreated subjects which received saline instead of the stimulant. A lower dose of physostigmine (0.3 mg/kg) did not significantly ($p>0.05$) alter motility induced by 6.0 mg/kg methylphenidate, and neither dose of physostigmine significantly ($p>0.05$) altered the effects of 12.0 mg/kg methylphenidate (Fig. 4). None of the other pretreatments applied in this study (neostigmine, scopolamine, methylscopolamine, or saline) significantly ($p>0.05$) changed methylphenidate-induced motility (Fig. 5).

Apomorphine. Motility induced by apomorphine (0.5 mg/kg) was significantly ($p<0.02$) reduced by pretreatment with physostigmine (0.6 mg/kg). Animals pretreated with physostigmine (0.6 mg/kg) and also injected with apomorphine (0.5 mg/kg) were not significantly different ($p>0.05$) from saline-injected animals pretreated with the same dose of physostigmine. Physostigmine blocked the apomorphine effect on motility during the entire observation period (Fig. 6).

DISCUSSION

The results presented above demonstrate that the cholinesterase inhibitor physostigmine antagonizes the effects of amphetaime, methylphenidate, and apomorphine on motility. Similar results have been reported for cholinergic drugs inhibiting amphetamine-induced stereotypy [3, 32, 47] and methylphenidate-induced stereotypy [12, 29, 49].

Comparison of the effects of physostigmine with an equimolar dose of neostigmine provides a means for distinguishing between the central and peripheral effects of cholinesterase inhibition. These drugs produce equivalent inhibition of acetylcholinesterase (AChE) activity at equimolar doses [35], but the low permeability of neostigmine to the blood-brain barrier results in little central effect of this drug

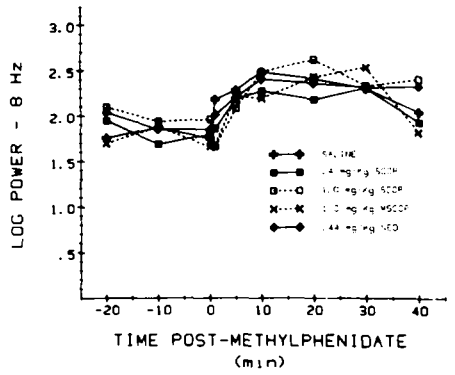

FIG. 5. Mean log power of 8 Hz movements in animals receiving 6.0 mg/kg methylphenidate and pretreated with either saline, scopolamine (SCOP), methylscopolamine (MSCOP), or neostigmine (NEO). Time before and after methylphenidate treatment is presented on the X-axis (injection at time zero). Pretreatments were administered just after the −20 minute period.

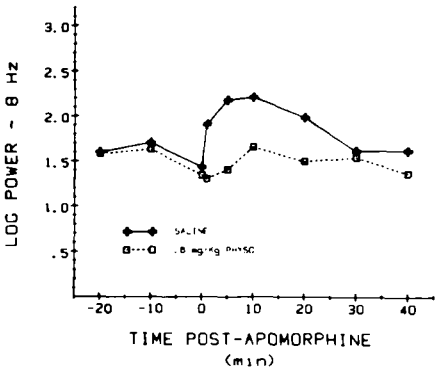

FIG. 6. Physostigmine inhibition of apomorphine-induced 8 Hz motility. The mean log power of 8 Hz movements is shown for animals receiving pretreatments of saline or physostigmine (PHYSO), followed by 0.5 mg/kg apomorphine. Time before and after apomorphine treatment is presented on the X-axis (injection at time zero). Pretreatments were administered just after the −20 minute period.

[33,42]. In this study, neostigmine (0.44 mg/kg) did not significantly alter methylphenidate-induced motility, at a dose equimolar to an inhibitory dose of physostigmine (0.6 mg/kg), indicating the central origin of the physostigmine effect.

A similar comparison was made of central versus peripheral cholinergic antagonism with scopolamine and methylscopolamine. Neither of these significantly altered methylphenidate-induced motility. In contrast, others have reported that cholinergic antagonists potentiate stimulant-induced gnawing [45] and sniffing [3]. The anticipated facilitation by scopolamine of stimulant-induced activity may have been obscured in the present study by a "ceiling" effect, since doubling the dose of methylphenidate did not result in any further increase in motility either (Fig. 4).

Of special interest in the present study, is the ability of physostigmine to inhibit the effects of stimulants acting at a postsynaptic (apomorphine) as well as at a presynaptic (amphetamine and methylphenidate) locus, suggesting that the primary effect may be postsynaptic to the dopamine projections which are believed to mediate stimulant-induced stereotypy [22, 27, 40]. This similarity between direct and indirect acting stimulants would not be predicted, given the assumption that cholinergic drugs alter stereotypy through a primary effect on sites presynaptic to these dopamine neurons.

Histochemical mapping studies of dopaminergic and cholinergic neurons suggest several possible sites of dopamine-acetylcholine interaction [28,36]. Among these are sites within both the nigrostriatal and mesolimbic dopamine pathways, including the striatum, substantia nigra, nucleus accumbens, and olfactory tubercle. Electrophysiological and pharmacological studies also suggest the existence of presynaptic cholinergic receptors on dopaminergic nigrostriatal neurons [13, 30, 38, 43] and on striato-nigro-thalmic projections in both the striatum and in substantia nigra zona reticulata [1, 13, 14, 50, 53]. These latter neurons are presumably non-dopaminergic, and may represent an important motor output pathyway through which nigral neurons influence motor behavior [54]. As such, this could be an important site for cholinergic modulation of dopamine-mediated motor behaviors.

Since drugs were administered systemically in the present study, it is not possible to conclude which neuroanatomical sites may mediate the observed effects, and it is likely that drugs used in this study act at several central sites. The observed effects then may indicate the net response of the systems mediating stereotypy to alterations at multiple cholinergic sites. While other interpretations of these data may be possible, the results reported here are consistent with the hypothesis that the major effects of cholinergic manipulation occur postsynaptically to the relevant dopamine pathways, with cholinergic mechanisms modulating or interacting with the more primary effects of dopaminergic system outflow downstream from the postsynaptic dopamine receptor. Since the site of this functional interaction may be important in the pathophysiology of several movement disorders [4, 13, 31], including Parkinsonism and Huntington's chorea, additional studies to localize the primary site of the reported dopamine-acetylcholine interaction are warranted.

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