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BRIEF COMMUNICATION

Reversal of a Mecamylamine-Induced Cognitive Deficit With the D_2 Agonist, LY 171555

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LEVIN, E. D., S. R. McGURK, J. E. ROSE AND L. L. BUTCHER. *Reversal of a mecamylamine-induced cognitive deficit with the D_2 agonist, LY 171555.* PHARMACOL BIOCHEM BEHAV **33**(4) 919–922, 1989.—Pharmacological blockade of either nicotinic or muscarinic cholinergic receptors has been found to impair choice accuracy in the radial-arm maze. Simultaneous blockade of both of these receptor types causes an additive impairment. However, despite these common effects, nicotinic and muscarinic receptors have been found to have differential involvement with dopamine receptors. The cognitive impairment caused by the muscarinic antagonist scopolamine is reversed by the D_1 antagonist SCH 23390 but is unaffected by the D_2 antagonist raclopride. In contrast, the cognitive impairment caused by the nicotinic antagonist mecamylamine is unaffected by SCH 23390 but is potentiated by raclopride. In the current study, the D_2 agonist LY 171555 was found to be effective in reversing the radial-arm maze choice accuracy impairment caused by mecamylamine. In contrast, the D_1 agonist SKF 38393 was not found to be effective. Thus, we have found selective dopaminergic D_1 and D_2 treatments which counteract the adverse cognitive effects of either nicotinic or muscarinic blockade. A combination of these treatments may be useful in treating the cognitive effects of generalized cholinergic underactivation.

Radial-arm maze Nicotinic Dopaminergic D_1 D_2 SKF 38393 LY 171555 Mecamylamine Agonist Antagonist

IN previous studies, we found that scopolamine-induced deficits in radial-arm maze choice accuracy could be reversed by dopamine (DA) receptor blockade (10,15). This reversal could also be seen after selective blockade of D_1 dopaminergic receptors (10). Selective D_2 blockade was ineffective. It seems that in cognitive as well as motor function there is a reciprocal interaction of DA and muscarinic acetylcholine (ACh) systems. Recently, it has also been shown that as with muscarinic ACh receptors, blockade of nicotinic ACh receptors impairs radial-arm maze choice accuracy performance (11). Furthermore, nicotinic and muscarinic blockade act together in an additive fashion in disrupting choice accuracy in the radial-arm maze (13). Despite this additive effect, however, muscarinic blockade and nicotinic blockade have quite different interactions with DA receptor antagonists. In contrast to the haloperidol-induced reversal of the scopolamine effect, haloperidol potentiates the adverse effect that mecamylamine has on radial-arm maze choice accuracy (16). This potentiation seems to be mediated via a different DA receptor subtype. Whereas D_1, but not D_2, receptor blockade reverses the choice accuracy deficit resulting from muscarinic antagonism, D_2, but not D_1, receptor blockade potentiates choice accuracy deficit resulting from nicotinic blockade (17). The present study was conducted to determine whether or not the mecamylamine-induced choice accuracy deficit in the radial-arm maze could be reversed by a selective D_2 agonist.

METHOD

Subjects

The ten female Sprague-Dawley rats (Bantin & Kingman Inc., Fremont, CA) used in the present experiment were housed singly in the test room on a reverse 12:12-hr light:dark cycle (lights came on at 1800). All behavioral testing was conducted during the dark phase in a dimly-lit room. The rats had ad lib access to drinking water but were maintained on a restricted feeding to keep their

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body weights at 80–85% of free-feeding levels adjusted for growth.

Apparatus

Behavioral testing of the rats was conducted on a radial 8-arm maze constructed of wood and plastic and was elevated 50 cm from the floor. The central platform was 87 cm in diameter and eight arms (10 × 69 cm) extended radially. A clear Plexiglas partition (32 cm long and 12 cm high) was attached to one side of the proximal end of each arm. Food cups for the reinforcements (2 cm in diameter and 1 cm deep) were located near the distal end of each arm. The maze was located in a testing room which contained many extramaze visual cues.

Behavioral Procedure

The rats were tested 4 days/week. Before each session each of the arms was baited with a ⅓ to ½ of a piece of sugar-coated cereal (Kellogg's Froot Loops). To begin the session the rat was placed in a circular plastic ring in the central platform. Then, after 10 seconds, the ring was lifted and the rat was allowed to move freely about the maze. Arm choices were recorded when the rat had placed all of its paws into the arm. Since the arms were not rebaited during the session, only the first entry into an arm was rewarded. Subsequent reentries were counted as errors. The session continued until the rat entered all eight arms or five minutes had elapsed, whichever came first. One measure of choice accuracy and one measure of choice latency were derived: the number of entries until an error was made (entries to repeat) and the total session latency divided by the number of arms entered (seconds per entry). Drug testing began after asymptotic levels of choice accuracy were achieved (at least 19 sessions).

Drug Administration

All rats received saline, the nicotinic antagonist mecamylamine (10.0 mg/kg), the dopamine D_1 agonist SKF 38393 (3 mg/kg), the D_2 agonist LY 171555 (0.05 mg/kg) and combinations of mecamylamine + SKF 38393 and mecamylamine + LY 171555, in a counterbalanced order. The drugs were mixed in 0.9% saline and injected (IP) in a volume of 1 ml/kg, twenty minutes before testing. Drugs were administered twice a week with at least two days between injections. On the day before each injection the rats were tested without drugs.

Data Analysis

The entries to repeat and seconds per entry measures were evaluated by analyses of variance for repeated measures. To further evaluate significant drug effects, planned comparsions using the Bonferroni correction for alpha slippage (19) were made for each of the drug conditions with saline and for each of the drug combinations with the individual drugs in the combination.

RESULTS

The choice accuracy measure, entries to repeat, clearly showed a reversal of the mecamylamine-induced deficit by coadministration of the D_2 agonist LY 171555 (Fig. 1). There was a significant overall drug effect on entries to repeat, $F(5,45) = 4.42$, $p<0.01$. Paired comparisons of the different drug treatments showed that the addition of the D_2 agonist LY 171555 to mecamylamine significantly attenuated the deficit caused by mecamylamine ($p<0.05$). On the other hand, the addition of the D_1 agonist SKF 38393 did not significantly change the mecamylamine-induced deficit. Neither LY 171555 nor SKF 38393 by itself caused significant changes in the number of entries to repeat, but there was a slight tendency towards a decrease in entries to repeat with LY 171555.

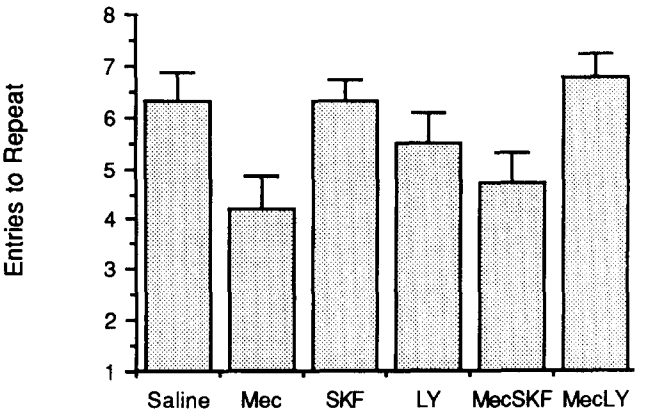

FIG. 1. Entries to repeat (mean ± standard error of the mean).

With choice latency there was also a reversal of the mecamylamine effect by coadministration of LY 171555 (Fig. 2). Again, there was a significant overall drug effect, $F(5,45) = 18.86$, $p<0.001$. Paired comparisons showed that all treatments except for SKF 38393 caused significant increases in choice latency relative to saline ($p<0.025$). Interestingly, even though both mecamylamine and LY 171555 by themselves each increased latency, there was a trend (albeit nonsignificant) toward LY 171555 attenuating the effect of mecamylamine on latency. However, with this combination latency was still significantly slower than with saline ($p<0.025$).

DISCUSSION

These results show that the mecamylamine-induced choice accuracy deficit in the radial-arm maze can be reversed by dopaminergic D_2 agonist treatment with LY 171555. This was the expected result inasmuch as we had observed in an earlier experiment that the selective D_2 antagonist raclopride potentiated the mecamylamine-induced choice accuracy deficit (17). Surprisingly, LY 171555 was also effective in attenuating the mecamylamine-induced increase in choice latency. When given alone, LY 171555, like mecamylamine caused an increase in choice latency.

A possible mechanism for the D_2 agonist reversal of the

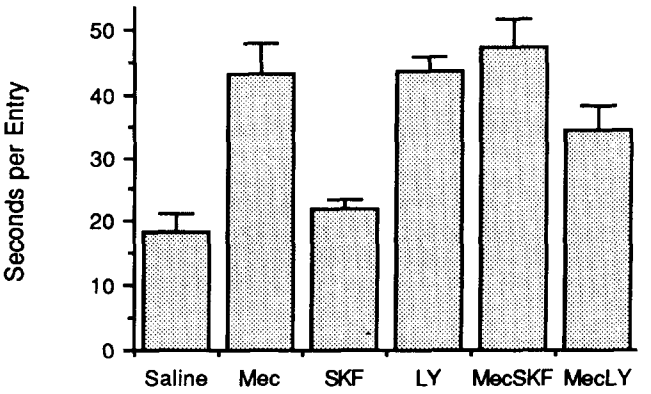

FIG. 2. Seconds per entry (mean ± standard error of the mean).

mecamylamine-induced deficits is the nicotinic innervation of DA neurons arising from the midbrain. There is a variety of neuroanatomical, electrophysiological and neurochemical evidence pointing to this interaction. Using intracerebral infusions of several tract-tracing agents in combination with choline acetyltransferase immunohistochemistry, Woolf and Butcher (21) and Gould, Woolf and Butcher (5) found cholinergic projections from the pedunculopontine and laterodorsal tegmental nuclei to both pars compacta and pars reticulata of the substantia nigra. Furthermore, Clarke and co-workers (3,4) found high concentrations of nicotinic receptors on DA cell bodies in the substantia nigra and ventral tegmental area (VTA) and on DA terminals in the striatum, nucleus accumbens and olfactory tubercle. Nicotinic stimulation seems to have excitatory influences on the activity of these DA cells. Systemically or locally applied nicotine has been found to excite DA neurons in the substantia nigra (2, 6, 14, 18). This excitation of the DA cells seemed to be specific in that it was blocked by mecamylamine (2,14). Systemically applied nicotine also has been found to increase the firing rates of DA cells in the VTA (6,18). This effect was substantially greater in terms of percent increase in firing rate than was seen in the substantia nigra (18). As in the substantia nigra, nicotine-induced increased firing rates in the VTA was reversed by mecamylamine (18). Mecamylamine applied systemically by itself decreased the firing rate of DA cells in the VTA (6). These findings suggest that there is a tonic excitatory nicotinic influence on DA cells in the midbrain.

Neurochemical findings supporting a nicotinic-dopaminergic interaction have been reported as well. Systemic nicotine administration has been found to increase DA turnover rates and HVA levels in the striatum (14). Ahtee and Kaakkola (1) found evidence that mecamylamine inhibits dopamine release from both striatal and mesolimbic pathways. Mecamylamine reduced the rate of α-MPT-induced depletion of DA in striatal and limbic areas. Mecamylamine was also found to reduce the probenecid-induced accumulation of the DA metabolite HVA in the striatum. Haikala and Ahtee (7) found that the nicotinic antagonists mecamylamine and pempidine decreased striatal concentration of 3-methoxytyramine which suggests that nigrostriatal DA neurons are controlled by a stimulatory nicotinic process. Imperato, Mulas and Di Chiara (8) used the microdialysis technique to examine the effects of nicotine on DA release in the striatum and nucleus accumbens of freely-moving rats. They found that nicotine administered systemically caused significant rises in DA concentrations in both the striatum and accumbens, with a greater effect in the accumbens (about a 100% increase) than in the striatum (about a 50% increase). Pretreatment with mecamylamine (1 mg/kg) completely blocked the nicotine effect on DA in the accumbens. With an in vitro preparation Rowell, Carr and Garner (20) found that nicotine induced DA release in the nucleus accumbens. This effect was partially antagonized by the nicotinic antagonist hexamethonium.

The mecamylamine effects in reduicng DA activity may be at least partially responsible for its adverse effects on choice accuracy. This could shed light on the mechanism by which a DA agonist is effective in counteracting the cognitive deficit caused by mecamylamine. Given that the D_2 agonist was found to effectively reverse the mecamylamine effect in the present experiment and that a D_2 antagonist was found to effectively potentiate the mecamylamine effect in a previous experiment, it seems likely that mecamylamine may express some of its amnestic actions via the D_2 receptor. The mesolimbocortical DA system may be particularly important in this nicotinic-DA interaction in cognitive function. The VTA has been found to be especially affected by nicotinic manipulations and it projects to forebrain areas such as the septum, hippocampus and frontal cortex which are crucial for adequate cognitive performance.

We previously found that D_1 blockade with SCH 23390 was effective in reversing the radial-arm maze choice accuracy impairment caused by the muscarinic receptor blocker scopolamine (10). With the present finding that the D_2 agonist LY 171555 reverses the deficit caused by nicotinic blockade, we now have treatments for the adverse cognitive effects of both types of cholinergic blockade. In that nicotinic and muscarinic blockade have been found to be additive in the cognitive deficit that they cause (13), and that disorders of cholinergic underactivation such as Alzheimer's disease probably involve both nicotinic and muscarinic receptors, the present results may be important in providing a drug combination which reverses the effects of generalized cholinergic underactivity.

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REFERENCES

1. Ahtee, L.; Kaakkola, S. Effect of mecamylamine on the fate of dopamine in striatal and mesolimbic areas of rat brain: Interaction with morphine and haloperidol. Br. J. Pharmacol. 62:213–218; 1978.
2. Clarke, P. B. S.; Hommer, D. W.; Pert, A.; Skirboll, L. R. Electrophysiological actions of nicotine on substantia nigra single units. Br. J. Pharmacol. 85:827–835; 1985.
3. Clarke, P. B. S.; Pert, A. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res. 348:355–358; 1985.
4. Clarke, P. B. S.; Pert, C. B.; Pert, A. Autoradiographic distribution of nicotine receptors in rat brain. Brain Res. 323:390–395; 1984.
5. Gould, E.; Woolf, N. J.; Butcher, L. L. Cholinergic projections to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. Neuroscience 28:611–623; 1989.
6. Grenhoff, J.; Aston-Jones, G.; Svensson, T. H. Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiol. Scand. 128:151–158; 1986.
7. Haikala, H.; Ahtee, L. Antagonism of the nicotine-induced changes of the striatal dopamine metabolism in mice by mecamylamine and pempidine. Naunyn Schmiedebergs Arch. Pharmacol. 338:169–173; 1988.
8. Imperato, A.; Mulas, A.; Di Chiara, G. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur. J. Pharmacol. 132:337–338; 1986.
9. Levin, E. D. Psychopharmacological effects in the radial-arm maze. Neurosci. Biobehav. Rev. 12:169–175; 1988.
10. Levin, E. D. Scopolamine interactions with D1 and D2 antagonists on radial-arm maze performance in rats. Behav. Neural Biol. 50:240–245; 1988.
11. Levin, E. D.; Castonguay, M.; Ellison, G. D. Effects of the nicotinic receptor blocker mecamylamine, on radial arm maze performance in rats. Behav. Neural Biol. 48:206–212; 1987.
12. Levin, E. D.; Galen, D.; Ellison, G. D. Chronic haloperidol effects on radial arm maze performance and oral movements in rats. Pharmacol. Biochem. Behav. 26:1–6; 1987.
13. Levin, E. D.; McGurk, S. R.; South, D.; Butcher, L. L. Effects of combined nicotinic and muscarinic blockade on choice accuracy in the radial-arm maze. Behav. Neural Biol. 51:270–277; 1989.
14. Lichtensteiger, W.; Hefti, F.; Felix, D.; Huwyler, T.; Melamed, E.; Schlumpf, M. Stimulation of nigrostriatal dopamine neurones by nicotine. Neuropharmacology 21:963–968; 1982.
15. McGurk, S. R.; Levin, E. D.; Butcher, L. L. Cholinergic-dopaminergic interactions in radial-arm maze performance. Behav. Neural Biol. 49:234–239; 1988.
16. McGurk, S. R.; Levin, E. D.; Butcher, L. L. Nicotinic-dopaminergic interactions in radial-arm maze performance in rats. Behav. Neural

Biol. 52:78–86; 1989.
17. McGurk, S. R.; Levin, E. D.; Butcher, L. L. Radial-arm maze performance in rats: Interaction of nicotinic blockade with D1 and D2 dopaminergic antagonists. Psychopharmacology (Berlin), in press; 1989.
18. Meru, G.; Yoon, K. P.; Boi, V.; Gessa, G. L.; Naes, L.; Westfall, T. C. Preferential stimulation of ventral tegmental area dopaminergic neurons by nicotine. Eur. J. Pharmacol. 141:395–399; 1987.
19. Neter, J.; Wasserman, W.; Kutner, M. H. Applied linear statistical models. 2nd ed. Homewood, IL: Richard E. Irwin, Inc. Publishers; 1985.
20. Rowell, R. P.; Carr, L. A.; Garner, A. C. Stimulation of [^{3}H]dopamine release by nicotine in rat nucleus accumbens. J. Neurochem. 49: 1449–1454; 1987.
21. Woolf, N. J.; Butcher, L. L. Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. Brain Res. Bull. 16: 603–637; 1986.