Pharmacology Biochemistry & Behavior, Vol. 15, pp. 285–288, 1981. Printed in the U.S.A.

Behavioral Effects of Deanol, of Hemicholinium and of Their Interaction

ROGER W. RUSSELL AND DONALD J. JENDEN[1]

Department of Pharmacology, School of Medicine and The Brain Research Institute
University of California, Los Angeles, CA 90024

Received 28 November 1980

RUSSELL, R. W. AND D. J. JENDEN. *Behavioral effects of deanol, of hemicholinium and of their interaction.* PHARMAC. BIOCHEM. BEHAV. 15(2) 285–288, 1981.—The present experiments were designed to study behavioral effects of two chemicals, which have opposite influences on the cholinergic neurotransmitter system, and of their interaction. It has been proposed that deanol is a direct precursor of acetylcholine (ACh) in brain and may enhance cholinergic transmission, while hemicholinium-3 (HC-3) acts to decrease ACh synthesis. Rats served as subjects. Doses of the drugs were based on results of earlier experiments; all were injected cerebroventricularly. The six treatment groups were: saline only; HC-3 (10 μg); HC-3 (10 μg) + deanol (1 μg); HC-3 (10 μg) + deanol (10 μg); deanol (1 μg); and deanol (10 μg). Behaviors measured were: reactivity to visual and tactile stimuli; resistance to capture and handling, vocalization, muscular tension; reactivity to non-contingent aversive stimulation; and, shock-induced defence reaction. With the exception of the defence reaction, all behaviors showed significant effects between the various drug treatments: deanol had no significant effect on the behaviors; animals receiving HC-3 only clearly showed responses which were enhanced above the levels of any of the other treatment groups; deanol had a dose-dependent effect of suppressing HC-3 enhanced behavior. The present results are consistent with the generalization that decreased cholinergic activity is associated with hyper-reactivity, and increased cholinergic activity with hyporeactivity. They indicate that the behavioral effects of deanol are dependent upon the state of the cholinergic system, interacting in combination with HC-3 but not alone.

Acetylcholine Cholinergic neurotransmitter system Deanol "Defense reaction" syndrome Hemicholinium-3 Reactivity

THE clinical use of dimethylaminoethanol (deanol) for the treatment of a variety of disorders, e.g., tardive dyskinesia, Huntington's chorea, has recently attracted increasing interest [2,14]. It has been proposed that deanol is a direct precursor of acetylcholine (ACh) in the brain and thus may enhance cholinergic neurotransmission [10]. With the opposite effect is hemicholinium-3 (HC-3) which acts to decrease ACh synthesis by inhibiting high affinity choline (Ch) uptake at the cholinergic nerve terminal [3,4]. It has been reported that interaction of the two drugs may result in the suppression by deanol of behavioral effects produced by HC-3 [5].

Earlier studies in our laboratory have shown a highly precise inverse relation between acute changes in ACh levels following cerebroventricular administration of HC-3 and behavioral hyper-reactivity, reactivity increasing in a dose dependent manner as dose of HC-3 increased and ACh levels decreased [3,12]. During the course of these experiments we also discovered that deanol is a common impurity in HC-3 and that, when present, it is capable of antagonizing the behavioral and other effects of HC-3 [5].

The present report describes a series of experiments in which commercial HC-3 was recrystallized to remove deanol in order to re-evaluate effects on behavior of deanol, of hemicholinium and of interactions between the two.

METHOD

Animals

Male Sprague-Dawley rats (200–250 g; Simonsen, Gilroy, CA) were used in all experiments. They were housed in individual cages in temperature and humidity controlled rooms with continuous light and were maintained on ad lib laboratory chow and a constant water supply. They were randomly assigned to six treatment groups, with six animals per group.

Stainless steel cerebroventricular cannulae were prepared and implanted as described by Russell and Macri [12]. Animals were maintained postoperatively for 5–6 days before experimental treatments began.

Measures of Behavior

Multiple stimulus rating scale. One of the three measures of behavior consisted of a standardized rating scale designed to evaluate the relative reactivity of animals during exposure to a variety of test conditions [3,7]. The exact procedure has been described in earlier reports from our laboratory [5,12]. Basically, six-point rating scales were used for each of five test conditions involving reactions to visual and tactile stimuli, resistance to capture and handling, vocalization and muscular tension. Total scores could range from 5 (hypo-

[1]Requests for reprints should be addressed to Dr. Donald J. Jenden, Department of Pharmacology, School of Medicine, University of California, Los Angeles, CA 90024.

reactive) to 30 (most hyper-reactive). All ratings were made by a trained experimenter who was not aware of the treatment to which an animal had been exposed. Checks were made of the consistency of ratings by different experimenters; no significant differences were found.

Reactivity. Animals were also exposed to a situation involving non-contingent aversive stimulation, i.e., unconditioned reactions (UR) to inescapable electric foot-shock. It is well established that low shock intensities produce a flinching response, followed at higher intensities by skeletal activity which is intensity dependent [8]. All shock intensities in the present experiments were above the threshold for the flinching reaction. Observations were made with the animals in a test chamber 30.5×30.5×30.5 cm, the top and sides of which were made of transparent plastic. Shock intensities were controlled by a Grason-Stadler shock generator and delivered to the stainless steel rods constituting the floor of the chamber through a scrambler which changed the polarity of the grid automatically. The duration of each shock pulse was 0.5 sec and shocks were delivered at a frequency of 20 per min. Shocks of different intensities were given in groups of 10 pulses in a counterbalanced order: 1.0, 2.0, 0.5, 4.0, 0.5, 4.0, 1.0, 2.0, 4.0, 2.0, 0.5, and 1.0 mA. Thus each test session involved reactions to 120 shock pulses, 30 at each of the four intensities. Records were kept as to whether or not each shock pulse stimulated a UR, i.e., jumping, prancing or running [8]. All trials were carried out in a room darkened except for a 60 W electric bulb directly over the test chamber. Extraneous sounds were masked by a continuous white noise background.

Shock-induced "defense reaction" syndrome. Responses other than hyper-reactivity are induced by foot-shock stimulation. One class of these, variously referred to as "defense reactions" [1], "reflexive fighting" [15], "aggression" [9] and "defensive threat" [16], is elicited in the presence of another animal and is evidenced by the assumption of a face-to-face "boxing posture". The syndrome has been described as ". . . stereotyped threat behaviors which frequently occur as precursors to more overt aggression and which are controlled by similar stimuli" [16]. It was decided to include the syndrome in the present experiments as a means of studying behavior which differed very obviously from reactivity as described above, yet could be produced under identical conditions of non-contingent aversive stimulation. Would the experimental treatments affect the two behaviors differentially?

Conditions for eliciting the defense reaction syndrome have been specified in detail [15]. The specifications were satisfied by the same apparatus and procedure used for studying reactivity as described above. However, the measure of behavior in this instance was the number of times the defense posture was assumed during each shock pulse. The same shock intensities were administered in the same counterbalanced order. Animals in each treatment group were paired randomly, providing three pairs for study under the test procedure.

Procedure

The basic research design was modelled after our earlier study of the antagonism by deanol of some behavioral effects of hemicholinium [5]. Animals were assigned randomly to one of six treatment groups: saline only, HC-3 (10 μg); HC-3 (10 μg) + deanol (1 μg); HC-3 (10 μg) + deanol (10 μg); deanol (1 μg); and deanol (10 μg). The various behaviors were measured on three consecutive days in the following order: multiple stimulus rating scale, reactivity, defense reaction. Day 1 measures established pretreatment baselines and Day 3 measures provided means of assessing recovery and possible carry-over effect. On Day 2 each animal received his respective treatment and 2 hr later underwent the behavioral assays.

TABLE 1

BEHAVIORAL EFFECTS OF DEANOL, HC-3 AND THEIR INTERACTIONS: PERCENT OF PRETREATMENT BASELINE

Treatment	Multiple-Stimulus Rating	Reactivity
	Mean S.E.M	Mean S.E.M
Saline	88.8 ± 5.59	84.7 ± 14.07
HC-3 (10)*	436.3 ± 33.79	192.0 ± 21.29
Deanol (1)	93.8 ± 3.92	103.7 ± 1.05
Deanol (10)	90.5 ± 4.27	99.3 ± 7.35
HC-3 (10) + Deanol (10)	140.0 ± 17.13	141.0 ± 13.43
HC-3 (10) + Deanol (1)	275.7 ± 41.08	175.7 ± 23.00

*Numbers in parentheses indicate μg.

All drugs were injected cerebroventricularly in a total volume of 2 μl. The particular doses used were selected on the basis of earlier research results as being within the effective dose ranges for at least some behavioral measures. HC-3 and deanol used in the present experiments were purchased from Eastman (Rochester, NY). HC-3 was recrystallized from hot ethanol/methanol (1:1) and dried *in vacuo* at room temperature before being used in the treatment phase of the experiments [5].

Analyses of the results were carried out using parametric statistics: ANOVAs when significant were followed by *t*-tests. Behavioral data were examined in terms of total responses during each assay period and of responses at each shock intensity for the measures of hyper-reactivity and defense reaction.

RESULTS

General Effects

One-way ANOVAs for treatment effects (Day 2) show statistically significant between-group differences for two of the behavioral parameters: multiple stimulus rating, $F(5,30)=37.61$, $p<0.01$, and reactivity, $F(5,30)=8.22$, $p<0.025$. There was no significant treatment-effect relation for the defense reaction, $F(5,12)=1.97$, $p>0.05$. Further analyses concentrated on the former two behavioral measures.

Effects of the various treatments are summarized in Table 1. Gross examination of the table suggests that the injection procedure itself tended to decrease responding in the saline groups. HC-3 appears to have enhanced responding and deanol seems to have had little effect.

Duration of Effects

Observations were repeated once on each of three days, the various experimental drug treatments being introduced

on Day 2. Had there been carry-over effects of a treatment or of repeated exposures to the behavioral assays, such effects would be expected to appear as differences between Day 1 and Day 3 measures. With but three exceptions among the 12 sets, t-tests established that such differences were not statistically significant: the behaviors had returned to pretreatment baselines within 24 hr after treatment. The exceptions were in measures of reactivity for the two groups receiving deanol only and for the HC-3 (10 μg) + deanol (10 μg) group: deanol (1 μg), $t(5)=3.83$, $p<0.01$; deanol (10 μg), $t(5)=3.59$, $p<0.01$; HC-3 (10 μg) + deanol (10 μg), $t(5)=2.85$, $p<0.025$. In all three cases responses were less on post than on pretreatment trials.

Effects of Deanol

The gross observation (Table 1) that deanol appeared to have little or no effect on the behaviors studied was confirmed by statistical analyses. ANOVAs showed no significant differences among measures for the two deanol groups and the saline control group: behavioral ratings, $F(2,15)=0.30$, $p>0.05$; reactivity, $F(2,15)=1.18$, $p>0.05$.

Effects of HC-3

Significant effects reflected in the overall ANOVAs were found among the treatment groups receiving HC-3. The rank order of mean levels of responding for the six groups was the same for measures of behavioral ratings and hyper-reactivity: (from low to high) saline; deanol (10 μg); deanol (1 μg); HC-3 (10 μg) + deanol (10 μg); HC-3 (10 μg) + deanol (1 μg); HC-3 (10 μg). The probability of random congruence of a rank order of six treatments is 0.0014. Student's t-tests of differences between the HC-3 (10 μg) and the two other high groups were significant for the comparisons with the HC-3 (10 μg) + deanol (10 μg) group (ratings, $t(5)=8.03$, $p<0.01$; reactivity, $t(5)=5.40$, $p<0.01$), but not for comparisons with the HC-3 (10 μg) + deanol (1 μg) group (ratings, $t(5)=2.86$, $p>0.05$; reactivity, $t(5)=0.37$, $p>0.05$). Animals receiving HC-3 only clearly showed responses which were enhanced above levels characteristic of any of the other treatment groups.

Interactive Effects

These analyses also provide evidence for the occurrence of interactive effects between HC-3 and deanol. Both dose levels of deanol produced some suppression of HC-3 enhanced behavior. The magnitude of suppression was dose-dependent for the behavioral ratings, deanol (10 μg) having a significantly greater effect than deanol (1 μg), $t(5)=4.54$, $p<0.01$. The trend for hyper-reactivity, although present, was not statistically significant, $t(5)=0.95$, $p>0.05$.

Relations to Shock Intensity

Analyses were carried out to examine possible relations between behavioral effects of the various experimental treatments and foot-shock intensities applied in measuring the reactivity and defence reaction behaviors. In all but one instance strong positive relations were found, i.e., response magnitudes increased as shock intensity increased. Trends were indicated by Spearman rank order correlation coefficients which ranged between +0.80 and +1.00. The only exception was in the saline group, whose level of defence reaction was low at all shock intensities.

DISCUSSION

The present experiments expand our earlier observations of behavioral consequences of pharmacological manipulation of cholinergic events in the central nervous system [11]. The present results are consistent with the generalization that changes in cholinergic activity are related to changes in behavioral reactivity. They corroborate the fact that decreased cholinergic activity is associated with hyper-reactivity [12] and increased cholinergic activity, with hypo-reactivity [13].

Present results also clearly illustrate differential effects of changes in cholinergic function on behavior, some behaviors being affected and others not. Two quite different behavioral patterns, i.e., reactivity and defence reaction, were elicited under identical stimulus conditions, the only environmental difference being the presence of a second animal during assay of the defence reaction. As other investigators have reported [1,15], the magnitudes of both these behaviors were positively correlated with shock intensity. Despite these similarities, decrease in cholinergic activity by HC-3 resulted in highly significant hyper-reactivity, while no differential effects of the drugs were observable in the defense reaction. In a manner of speaking, the former appears to be "cholinergically coded" and the latter, not.

Present results indicate that behavioral effects of deanol at the doses used were dependent upon the state of the cholinergic system. Administered by itself in otherwise untreated animals deanol produced no changes in any of the three behaviors which differed from injections of saline. However, when injected cerebroventricularly in combination with HC-3 deanol at the two dose levels suppressed HC-3 induced hyper-reactivity as measured both by the behavioral rating scale and in the non-contingent aversive situation.

Possible neurochemical mechanisms underlying the selective effects of deanol on behavior have been considered [5,6]. The most likely explanation appears to be an elevation of choline levels in the brain following deanol, perhaps as a result of competition for an exit pathway; Ch is well known to compete with hemicholinium [17], and has been reported to have agonist properties of its own [18].

ACKNOWLEDGEMENTS

This research was supported by USPHS grant MH17691. We wish to give special acknowledgement for the technical assistance of the late J. R. Macri. The editorial contributions of Flo Comes during preparation of the manuscript are greatly appreciated.

REFERENCES

1. Bolles, R. C. Species-specific defense reactions. In: *Aversive Conditioning and Learning*, edited by F. R. Brush. New York: Academic Press, 1971, pp. 183–233.
2. Casey, D. E. and D. Denny. Pharmacological characterization of tardive dyskinesia. *Psychopharmacology* **54:** 1–8, 1977.
3. Freeman, J. J., J. R. Macri, R. L. Choi and D. J. Jenden. Studies on the behavioral and biochemical effects of hemicholinium *in vivo*. *J. Pharmac. exp. Ther.* **210:** 91–97, 1979.

4. Guyenet, P., P. Lefresne, J. Rossier, J. Beaujouan and J. Glowinski. Inhibition by hemicholinium-3 of [^{14}C] acetylcholine synthesis and [^{3}H] choline high affinity uptake in rat striatal synaptosomes. *Molec. Pharmac.* **9:** 630–639, 1973.
5. Jenden, D. J., J. Macri, M. Roch and R. W. Russell. Antagonism by deanol of some behavioral effects of hemicholinium. *Communs Psychopharmac.* **1:** 575–580, 1977.
6. Jope, R. S. and D. J. Jenden. Dimethylaminoethanol (deanol) metabolism in rat brain and its effect on acetylcholine synthesis. *J. Pharmac. exp. Ther.* **211:** 472–479, 1979.
7. King, F. A. Effects of septal and amygdaloid lesions on emotional behavior and conditional avoidance responses in the rat. *J. nerv. ment. Dis.* **126:** 57–63, 1958.
8. Meyer, J. S. Some effects of noncontingent aversive stimulation. In: *Aversive Conditioning and Learning*, edited by F. R. Brush. New York: Academic Press, 1971, pp. 464–536.
9. Moyer, K. E. *The Psychobiology of Aggression.* New York: Harper and Row, 1976.
10. Peiffer, C. C., E. H. Denny, W. Gallagher, R. P. Smith, W. Bevan, Jr., K. F. Killam, E. K. Killam and W. Blackmore. Stimulant effects of 2-dimethylaminoethanol—possible precursor of brain acetylcholine. *Science* **126:** 610–611, 1957.
11. Russell, R. W. Cholinergic substrates of behavior. In: *Cholinergic Mechanisms and Psychopharmacology*, edited by D. J. Jenden. New York: Plenum Press, 1977, pp. 709–731.
12. Russell, R. W. and J. R. Macri. Central cholinergic involvement in behavioral hyper-reactivity. *Pharmac. Biochem. Behav.* **10:** 43–48, 1979.
13. Russell, R. W., R. Pechnick and R. S. Jope. Effects of lithium on behavioral reactivity: relation to increases in brain cholinergic activity. *Psychopharmacology* **73:** 122–125, 1981.
14. Simpson, G. M., A. Voitashevsky, M. A. Young and J. H. Lee. Deanol in the treatment of tardive dyskinesia. *Psychopharmacology* **52:** 257–261, 1977.
15. Ulrich, R. E. and N. H. Azrin. Reflexive fighting in response to aversive stimulation. *J. exp. Analysis Behav.* **5:** 511–520, 1962.
16. Ulrich, R. E. and B. Symannek. Pain as a stimulus for aggression. In: *Aggressive Behavior*, edited by S. Garattini and E. B. Sigg. New York: Wiley, 1969, pp. 59–69.
17. Krnjevic, K. and W. Reinhardt. Choline excites cortical neurons. *Science* **206:** 1321–1322, 1979.
18. MacIntosh, F. C. and B. Collier. Neurochemistry of cholinergic terminals. In: *Handbook of Experimental Pharmacology, Vol. 42, Neuromuscular Junction*, edited by E. Zaimis. New York: Springer-Verlag, 1976, pp. 99–228.