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BRIEF COMMUNICATION

Inability of Hexamethonium to Block the Discriminative Stimulus (S^D) Property of Nicotine[1]

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HAZELL, P., D. W. PETERSON AND R. LAVERTY. *Inability of hexamethonium to block the discriminative stimulus (S^D) property of nicotine.* PHARMAC. BIOCHEM. BEHAV. 9(1) 137–140, 1978.—Rats were trained to discriminate between levers on a white or black wall to obtain food reinforcement, using nicotine or saline administration as the discriminative stimulus (S^D). When hexamethonium was administered, either peripherally or intraventricularly, before the nicotine injection these rats responded as though they had received nicotine alone. This indicates that nicotine receptors responsible for its S^D property are not blocked by hexamethonium, or alternatively that it is necessary to block the peripheral and central actions simultaneously to completely eliminate the cueing effect of the nicotine injection.

Nicotine Hexamethonium Discriminative stimulus Operant behaviour Intraventricular

DRUGS are capable of gaining stimulus control of operant behaviour, so that animals give one response in the presence of one drug and a second response in the presence of another drug [6]. In a drug-saline discrimination a novel drug, with actions similar to the drug used in the discrimination training, will elicit a drug response; whereas a drug or drug combination with dissimilar actions will result in saline or random responding on the discrimination task. Using this type of experiment, it has been shown that when nicotine is given in combination with antagonists that do not cross the blood-brain barrier, such as hexamethonium or chlorisondamine, the subject still make a "nicotine" response. In contrast, when nicotine is given in combination with mecamylamine, a secondary ammonium compound which readily crosses into the brain, a saline response is evoked [5,8]. Another study [7] has shown that animals trained with subcutaneously administered nicotine make a nicotine response when tested with intraventricular nicotine, and vice versa. It has, therefore, been concluded that the discriminative stimulus (S^D) properties of nicotine involve an action of the drug on the central nervous system (CNS). On the basis of these findings, it would seem a reasonable assumption that if the CNS activity of a S^D drug such as nicotine was blocked, but the peripheral activity left intact, the cueing properties would be blocked. This assumption is tested in the following experiments using nicotine and intraventricular hexamethonium as an antagonist of the CNS effects [1].

METHOD

Animals

Ten male Wistar rats, 6 months old, and weighing 300–350 g before food deprivation, were used. They were housed in individual cages in a temperature controlled room, and kept on a natural day/night cycle. Throughout the training and testing period, the animals were maintained at 80–85% of the normal body weights by controlled daily feeding at the end of the afternoon behavioural sessions. Part way through the testing period all the rats were implanted with permanent intraventricular cannulae, while anaesthetized with pentobarbitone, 25 mg/kg, and ketamine, 80 mg/kg. The cannulae were made by drilling a hole through a brass electrical screw and gluing a piece of polyethylene tubing (o.d. 1.0 mm) into this hole so that one end protruded 3.75 mm beyond the flat surface of the head of the screw. The cannulae were implanted 1.5 mm lateral and 0.5 mm posterior to the bregma, with the head of the screw flush against the skull, so that the tip of the polyethylene tubing would be in the right lateral ventricle. A 26 ga needle fit snugly into the polyethylene tubing for the infusion of drugs intraventricularly.

Apparatus

BRS/LVE equipment was used. A rodent test cage was modified by painting one side wall black and the other side wall white. A lever extended through each of the side walls, 4 cm from the grid floor and 7 cm from the clear plastic door in the front of the cage. These levers will subsequently be referred to as the black lever and the white lever. In the center of the back wall was a food cup connected to a pellet dispenser for the programmed delivery of 45 mg food pellets (P.J. Noyes Co., Lancaster, NH). The only light source was a white house light on the back wall of the test cage. Pro-

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gramming and recording equipment was situated on a shelf below the cage.

Procedure

Discrimination training. On Days 1 and 2, half the rats were shaped to press the white lever and half were shaped to press the black lever. In both cases the alternative lever was withdrawn. The animals were required to make a minimum of 30 responses. On Day 3 the animals were required to make 30 responses on the alternative lever with the original lever withdrawn. In the morning of Day 4 the rats again made 30 responses on the original training lever, in the afternoon 30 responses on the alternative lever. On Day 5 the procedure was the same as Day 4 except that a 10 sec time out after each lever press was incorporated in the schedule. After 3 sec of this time out period the house light was turned off and came on again after 7 sec to signal that the bar was operative. On Day 6, three of the rats originally shaped on the white lever, and two of those shaped on the black lever were designated to Group A, the remainder became Group B. This was done to counteract any bar preference effects produced by the shaping procedure. From Days 7 to 19, the animals received morning and afternoon discrimination training sessions. In these sessions both white and black levers were present. Pressing the correct lever (determined by whether the animals had been given a 1 ml/kg injection of nicotine (0.4 mg/kg) or NaCl (9 g/l) 10 min before the session) was rewarded with a food pellet and timed out both levers for 10 sec. The house light turned off after 3 sec and remained off for another 7 sec. Pressing the incorrect lever was not rewarded, timed out both levers for 10 sec and turned the house light off for the same time. For Group A the white lever was correct following a nicotine injection, the black lever correct following NaCl. The converse was true for Group B. The drug condition for each session was randomized with the constraint that no one drug condition was repeated for more than two consecutive sessions. The sequence for the first 12 sessions, for example, was: N S N S N S S N S N N S where N=nicotine and S=saline. These sessions were initiated by placing each animal in the test cage facing the feeder tray (which contained one pellet) while both levers were timed out for 10 sec and the house light remained off for this period. The first lever pressed by the animal at the end of the interval was recorded as the first trial choice (FTC). By Day 19 all animals were able to make four correct FTCs in five consecutive sessions. This was the criterion set for learning the discrimination task.

Testing. Testing sessions and regular discrimination training sessions were held in the mornings of alternate days after Day 19, except when the group failed to achieve criterion performance on a training day (animals were considered to have maintained criterion if 80% or more of the group made the correct FTC). On the two occasions that the groups failed to achieve criterion they received another training session on the following day, and achieved criterion in this session. The drug designation for training sessions was determined on the same basis as the discrimination sessions prior to Day 19. This procedure was interrupted for a week following Test 2, at which time permanent indwelling cannulae were implanted in the subjects, then given 3 days to recover, and another 3 days of retraining to criterion (one session daily). Three animals died of post-operative respiratory depression, despite artificial resuscitation. One other animal was given resuscitation but it subsequently performed to criterion and was included in the tests that followed. As the experimental series proceeded several more animals fell ill and three died. These animals were treated with chloramphenicol and were not included in the tests. Post mortems of the dead rats showed purulent exudate involving the region around the cannula tip. At the conclusion of the series the remaining rats were killed and checked for cannula placement. The cannulae were in the lateral ventricles in all cases.

In test sessions the animals were placed in the test cage facing the feeder, which contained one food pellet. The house light remained off for 10 sec. When it came on again the first lever the animal pressed was recorded and the animal was then immediately removed from the chamber and returned to its home cage. The pressing of either lever was of no other consequence. All peripheral drug administrations were in volumes of 1 ml/kg unless otherwise stated, and drugs were made up in saline solution. Subcutaneous SC injections were given in the skin fold above the scapula. Intraventricular (ICV) drugs were delivered in volumes of 20 μl over 60 sec using a Harvard infusion/withdrawal pump. The infusion needle was always left in place for another 30 sec. The ICV hexamethonium was made up in deionized water (pH range 6.8–7.0).

In Test 1, the animals were given hexamethonium bromide 1.0 mg/kg SC 20 min before the test. In Test 2, the animals were given a similar injection of hexamethonium followed 10 min later by nicotine hydrogen tartrate 0.4 mg/kg SC. The test was given 10 min after the nicotine injection. In Test 3, NaCl 9 g/l was given ICV followed immediately by nicotine 0.4 mg/kg. The test was given 10 min later. In Test 4, hexamethonium 10 μg was given ICV followed by nicotine SC, and tested 10 min later. A similar test was performed in the next testing session (repeated testing was necessary because the animal numbers were decreasing). In Test 5, animals received hexamethonium 10 μg ICV followed by NaCl 9 g/l SC. The test was given 10 min later. Test 5 was also repeated. In Test 6, the animals were given 0.4 mg/kg nicotine dissolved in a 1% (10 g/l) solution of lignocaine administered SC in a volume of 1 ml/kg, and tested on the discrimination task 10 min later.

RESULTS

A summary of the results can be found on Table 1. After training, when all rats were discriminating nicotine from saline at above criterion levels, test 1 was run. This test shows that when hexamethonium was injected SC, the rats responded as though they had received saline. This same hexamethonium injection with nicotine failed to block the nicotine cue, indicated by the significant nicotine response in test 2. Similarily, test 4 shows that hexamethonium administered ICV before peripheral nicotine also results in a significant nicotine response. The controls for the ICV injection, tests 3 and 5, did not produce responding that can be statistically characterized as saline or nicotine-like, which may indicate a disruptive effect of the ICV injection on the discrimination. Finally, test 6 eliminates the possibility that peripheral nociceptor stimulation is responsible for the nicotine cue because a nicotine response was still obtained when 1% lignocaine was added to the injection.

DISCUSSION

The most interesting finding in this study was that

TABLE 1

NICOTINE RESPONSE OF RATS ON NICOTINE-SALINE DISCRIMINATION

Drugs	No. Animals	No. Test	Fraction giving nicotine response
1. Hexamethonium 1.0 mg/kg SC	10	10	2/10†
2. Hexamethonium 1.0 mg/kg SC Nicotine 0.4 mg/kg SC	10	10	8/10‡
3. NaCl 9 g/l ICV Nicotine 0.4 mg/kg SC	7	7	5/7
4. Hexamethonium 10 μg ICV Nicotine 0.4 mg/kg SC	6	11	7/9*‡
5. Hexamethonium 10 μg ICV NaCl 9 g/l SC	5	8	3/7§
6. Nicotine 0.4 mg/kg SC Lignocaine 10 g/l SC	3	6	5/6‡

* two rats failed to respond
§ one rat failed to respond
† Significantly different from nicotine, Chi-Squared, $p<0.05$
‡ Significantly different from saline, Chi-Squared, $p<0.05$

hexamethonium (ICV) failed to block the discriminative cueing effect of nicotine SC, so that the animals still made drug responses. There are three possible explanations for this finding: 1. The nicotine S^D is of peripheral origin; 2. the nicotine S^D is of central origin but is not blocked by the antagonist; 3. the nicotine S^D is a stimulus complex of peripheral and central origin which generalizes to a peripheral-only or central-only activity of the drug.

Experimental evidence against a peripheral interoceptive cue site for nicotine has already been reviewed in the introduction. The possibility that the S^D was arising from some peripheral nicotinic action that is not antagonized by ganglion blockers was considered. The results of Test 6 seem to discount the idea that local nociceptor stimulation provides the cue. That nicotine produces its cue by some action on the neuromuscular junction is unlikely, but could be tested by giving nicotine in conjunction with a neuromuscular blocker in a transfer test.

The second possibility is that nicotine may be producing its cueing effect by some CNS action that is not antagonized by hexamethonium. An obvious limitation of this study is that a dose range of central hexamethonium was not tested. However, the efficacy of hexamethonium 10 μg ICV in preventing convulsions induced by nicotine 3.0 mg/kg was proven in a pilot study to these experiments. Mecamylamine, due to its secondary amine structure, rapidly penetrates the CNS and has been shown to be an effective blocker of the nicotine cue [5,8]. Why mecamylamine should block the nicotine while centrally administered hexamethonium does not, poses an interesting question. Mecamylamine has been shown to have a presynaptic action in blocking acetylcholine release in the rabbit superior cervical ganglion [4], an action which hexamethonium does not have. Perhaps the nicotine cue is produced by a presynaptic release of acetylcholine within the CNS. Alternatively, the nicotine cue may be produced at some site within the CNS that is not accessible to ICV hexamethonium. Interestingly, when we studied mecamylamine on nicotine induced convulsions, peripheral mecamylamine (6 mg/kg) prevented all signs of nicotine poisoning while central mecamylamine (6 μg) had no effect (unpublished observations). It is tempting to suggest that sites of action of the nicotine cue and nicotine induced convulsions are anatomically distinct, one being accessible to peripheral mecamylamine the other being accessible to central hexamethonium. However, it is known that hexamethonium does distribute fairly evenly within the CNS [2], and that mecamylamine, with greater ability to pass membranes, would be expected to distribute at least as widely as hexamethonium. One possible explanation of the lack of control of nicotine convulsions by mecamyline, ICV, is that a rapid loss of the drug via the choroid plexus may prevent an effective concentration from being achieved in the neuronal tissue.

The third possibility is that the nicotine interoceptive cue is a stimulus complex arising from both the central and peripheral actions of nicotine. This explanation is consistent with the 'multidimensional' model proposed by Barry [3] for the S^D properties of drugs. This possibility is consistent with the findings made here that hexamethonium, administered either centrally or peripherally, does not block the nicotine interoceptive cue. This model predicts that if hexamethonium was given centrally and peripherally simultaneously in conjunction with nicotine, the cueing effect would be blocked and the subjects would make saline or random responses.

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