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Intracranial Alpha-Methyl-P-Tyrosine and Response for Electrical Brain Stimulation[1,2]

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BROWN, S. L. AND R. FIAL. *Intracranial alpha-methyl-p-tyrosine and response for electrical brain stimulation.* PHARMAC. BIOCHEM. BEHAV. 3(4) 641–646, 1975. – Chemitrodes, allowing electrical and chemical stimulation of the brain at the same site, were implanted in 18 rats aimed at the medial forebrain bundle of the lateral hypothalamus. After these animals were trained to bar-press for electrical brain stimulation, the crystalline form of alpha-methyl-para-tyrosine (AMPT), an inhibitor of catecholamine synthesis, was administered intracranially, and change in response rate was noted. Intracranial tyrosine administration was also tested as a control study. It was found that AMPT depressed rate of response for intracranial self-stimulation, whereas tyrosine administered intracranially exhibited no such effects. This result lends support to data reported in the literature on the use of AMPT administered intraperitoneally or orally, and suggests a noradrenergic or dopaminergic system of reward in the lateral hypothalamus.

Alpha-methyl-para-tyrosine Self-stimulation Medial forebrain bundle Intracranial administration Norepinephrine Dopamine

RECENTLY much research has been done on the biochemical correlates of the electrical brain stimulation pathway postulated by Olds [17]. It has been found that the medial forebrain bundle contains monoaminergic fibers [7], and that both catecholamine and indoleamine transmitters are released during intracranial stimulation (ICS) [1,22]. Research in this area has dealt primarily with selective suppression of first the catecholamine levels of the brain and then the serotonin level, and notation of change in the response rate of the animal for self-stimulation. However, because this research has concentrated on peripheral administration of centrally-acting drugs, it has been difficult to separate the gross behavioral effects of the drugs from their neuronal effects (i.e. it is hard to determine whether a drop in response rate is due to general sedation of the animal or to localized activity in the brain). An exact determination of the roles of norepinephrine, dopamine, and serotonin in the mediation of reward will only be possible when these effects can be differentiated.

Various drugs are avilable which allow alteration of these neurochemical levels. Alpha-methyl-para-tyrosine (AMPT), shown to be a tyrosine hydroxylase inhibitor *in vitro* [16] and *in vivo* [21], has been extensively investigated in connection with the ICS pathway. AMPT blocks the biochemical pathway leading from phenylalanine to norepinephrine at the synthesis of dopa from tyrosine. After AMPT administration alpha-methyl-dopamine and alpha-methyl-norepinephrine are metabolized competitively along with small amounts of dopamine and norepinephrine [6,23], and normal dopamine and norepinephrine levels are suppressed. Thus this drug causes catecholamine depletion with no concomitant effects on brain serotonin level.

Poschel and Ninteman [18] have demonstrated that AMPT administered intraperitoneally suppresses response rate for ICS in the medial forebrain bundle of the posterior lateral hypothalamus of the rat, while methamphetamine hydrochloride causes reinstatement of the response. The degree of response suppression after AMPT administration appears to be dependent upon electrode placement as well as drug dosage [5]. Black and Cooper [3] have used both AMPT and DL-para-chlorophenylalanine (pCPA, a serotonin depletor) to selectively depress catecholamine and serotonin levels in the brain of the rat. Their results show a decrease in response rate after oral AMPT administration and no effect after pCPA administration across two independent reward tests. Poschel and Ninteman [19], however, have shown that pCPA may cause an increase in response for ICS at loci along the monoaminergic fibers ascending from the midbrain to the forebrain, whereas para-chloroamphetamine causes suppression of self-stimulation. From this they have postulated that the adrenergic fibers of the medial forebrain bundle subserve an excitatory

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system and that the serotonergic fibers subserve an inhibitory system.

The above experiments used intraperitoneal or oral administration of AMPT. In the experiment reported in this paper, DL-alpha-methyl-para-tyrosine was administered intracranially in crystalline form, a method which has been shown with various drugs to cause the greatest local effect and the least diffusion [12,13]. This technique was used because it was felt that the effects of AMPT administered by other routes could have been due to the peripheral effects of the drug, or to influences exerted in other parts of the brain. By localized administration to the neuronal pathway being studied, it is possible to exert some control over the radius of drug influence. It was felt not only that the toxic properties of large AMPT dosages could be avoided by an intracranial dosage method, but that the sedative effect upon rats sometimes reported for AMPT [10, 14, 15, 20, 21] might also be averted. If the reward system in the lateral hypothalamic portion of the medial forebrain bundle is coded monoaminergically, it would be expected that intracranial administration of AMPT to that area would cause depression of the ICS response rate, whereas if the observed effects of AMPT reported in the literature are due to the peripheral effects of the drug, then no change in response rate would occur.

Tyrosine was chosen as a control substance, since this organic chemical can act as a control for the mechanical and ionic artifacts of the methods employed. Because tyrosine hydroxylase is the rate-limiting factor in norepinephrine synthesis [11], since the hydroxylation of tyrosine to dopa is the corresponding rate-limiting step, addition of tyrosine to an area of concentrated catecholamine fibers would not be expected to cause a rise in response rate. Rather, it was expected that the tyrosine would be absorbed as free substrate for the tyrosine hydroxylase, and it was thought that this excess quantity of amino acid in the hypothalamus would cause no disruption in the functioning of the brain, and would thereby act as a control for the administration of the same amount of tyrosine α-methyl analogue.

METHOD

Animals

Eighteen experimentally naive male Long-Evans rats, weighing between 300 and 400 g each, were orginally implanted with permanent monopolar chemitrodes (devices which allow chemical and electrical stimulation of the brain at the same site). The chemitrodes were implanted at 1.2 mm lateral to the midline at Bregma and 8.5 mm below the skull surface with Bregma and Lambda leveled, and were aimed at the medial forebrain bundle of the dorsal lateral hypothalamus. All operations were performed under 50 mg/ml Nembutal anesthesia at 35 mg/kg intraperitoneally. The experimental animals were housed separately in non-automated wire cages, with food and water available ad lib.

Chemitrodes

Each chemitrode was constructed of a Yale 22 ga X 1 1/2 in. disposable hypodermic needle, coated with General Electric 9637 Insulating Varnish and cut to a 14 mm length. Externally, the chemitrode was soldered to a piece of insulated stainless steel wire which was connected to one pin of a two prong electrode plug. The other pin was soldered to a stainless steel screw mounted in the skull of the rat so as to provide a ground source. A Vita nondisposable stainless steel 28 ga X 1 in. hypodermic needle, cut to a 14.5 mm length and bent at the top, fit tightly into the hollow outer chemitrode and was used for the actual drug administration.

Histology

At the conclusion of this experiment all animals were sacrificed with an overdose of Nembutal. They were then perfused with isotonic saline and 10% Formalin, and their brains were removed. Each brain was frozen, 50 μ slices were cut by microtome, and the sections were mounted on glass slides and stained with cresyl-violet. All histologies verified the dorsal lateral hypothalamic placement at the level of the medial forebrain bundle. Figure 1 presents sample histological sections from 3 of the experimental animals, showing electrode placement.

Apparatus

The testing apparatus was a modified Skinner box, 26 X 26.5 X 49.5 cm in area. Three of the walls were made of plastic, and the front hinged wall was constructed of clear Plexiglas. The floor consisted of steel bars, 1 cm dia. and spaced 3 cm apart to allow urine and feces to fall through to the tray of sawdust underneath. A swivel mounted in the ceiling of the chamber held the electrode lead and allowed the animal free movement. The plastic lever measured 4.5 X 5 cm, and was mounted in the wall.

The entire chamber was mounted in a refrigerator, which, when closed, protected the rat from distractions in the surrounding environment without impairing ventilation. White noise was provided during all experimentation.

All programming was done automatically through a BRS solid-state programming unit. Each time the animal pressed the bar it received one stimulation, consisting of a 100 Hz 50 percent duty cycle square wave pulse train of 0.5 sec duration. Responses were recorded automatically on a Gerbrands cumulative recorder.

Procedure

One week after surgery each animal was placed in the experimental chamber and connected to the stimulating cable by the electrode plug mounted in its head. Current was then varied while free stimulations were given until the animal began to show behaviors characteristically associated with ICS, such as sniffing in the corners of the cage and heightened activity level. At this point the door of the refrigerator was closed and the animal was allowed access to the bar on a continuous reinforcement (CRF) schedule for 1 hr, after which it was removed from the testing chamber. Twenty-four hours later the animal was replaced in the box and tested for 1 hr while the current was adjusted for highest response rate. If the animal failed to learn the response by the end of this session it was discarded. On the third day the animal was tested with the voltage held constant, and response rate was taken as the baseline rate. Current levels ranged from 210–470 μA, depending upon the level of voltage that elicited ICS response behavior from each animal. On the fourth day the CRF performance was measured for 1 hr. Four hours later DL-alpha-methyl-para-tyrosine was administered, followed by 1 hr of CRF testing. Subse-

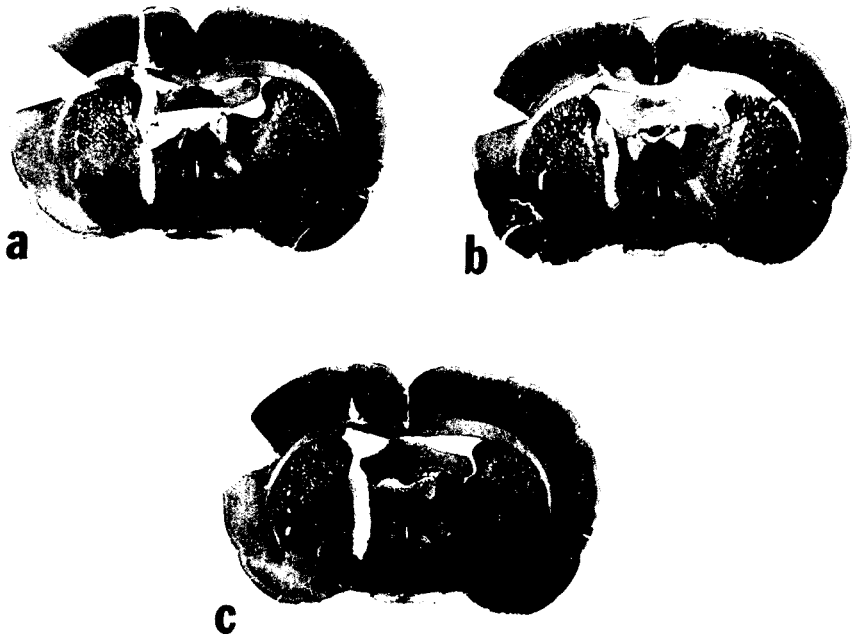

FIG. 1. Sample sections from the histologies of three rats. All tissue was prepared with cresyl violet stain. Section a was taken from Rat 1T (25 tap dosage), Section b was taken from Rat 1F (50 tap dosage), and Section c was taken from Rat 4S (75 tap dosage). Each of the above sections is a representative sampling of all histologies done in the particular dosage group, and in each case the placement of the electrode tip is at the dorsal lateral hypothalamus.

quently, the animal was measured on CRF for 1 hr every fourth hour. Sixteen hours after AMPT administration this procedure was changed to 1 hr every 16 hr, until 48 hr after resumption of the baseline rate. At this point the animal was tested for 1 hr again, and 4 hr later tyrosine was administered. Testing then continued every 4 hr for 16 hr, and every 16 hr subsequently for 1 week as outlined above. The animals were returned to their home cages between the one-hour test sessions.

Nine of the 18 rats were tested in the manner described. The other 9 were subject to identical procedures except that they received the tyrosine administration first, followed by the AMPT after one week.

Drug Administration

DL-alpha-methyl-para-tyrosine was used in crystalline form, as was tyrosine. Each drug was spread on a glass slide at a uniform thickness of 2 mm, and the 28 ga inner cannula was held upright and tapped onto the flat surface. This method has been reported to yield a uniform quantity of drug per tap [2,8]; therefore it was not necessary to compute the exact weight of each dosage level, although all 3 dosages were weighed on a Beckman balance and were determined to fall between 1 and 5 μg. Administration of the drug consisted simply of inserting the inner cannula into the chemitrode. The bend in the top of the cannula prevented it from slipping down into the brain. The cannula was purposely cut 0.5 mm longer than the outer chemitrode so as to break up necrotic tissue at the tip of the chemitrode. All cannulae were left in place during the entire experiment, and were only removed for approximately 4 min to allow for the preparation of the second drug, after which they were inserted for the remainder of the experiment. During the time that the cannula was removed from the animal, it was examined microscopically to determine whether or not all of the drug had disappeared. It has been reported that drug dosages in the amount used in this study diffuse out of a cannula of this size within 4 to 5 min [9].

Three different drug dosages, 25 taps, 50 taps, and 75 taps, were used. The 18 animals were divided into these 3 dosage groups with 6 rats in each group. Each animal received the same quantity of each drug.

RESULTS

DL-alpha-methyl-para-tyrosine, administered intracranially, produced a significant decrease in the self-stimulation response rate in each of the animals. A two-tailed Wilcoxon rank sum test showed a significant effect ($p<0.0046$) after drug administration. No relationship was shown between drug dosage and response rate, although latency until reinstatement of baseline rate proved to be a function of the amount of drug administered ($p<0.0046$ by the Wilcoxon rank sum test). Tyrosine produced no effect on response rate. Figures 2, 3, and 4 show the data from, respectively, the rats receiving the 25, 50, and 75 tap drug dosages. From these data it appears that AMPT causes an initial decrease in response rate which begins by Hour 4, becomes very marked by Hour 8, and by Hour 16 response is almost completely suppressed. The 25 tap dosage group had a uniform resumption of baseline rate at Hour 208 (Day 10 after drug administration), while the 50 tap dosage group varied between Hours 224–240 (Days 10–11), and the 75 tap

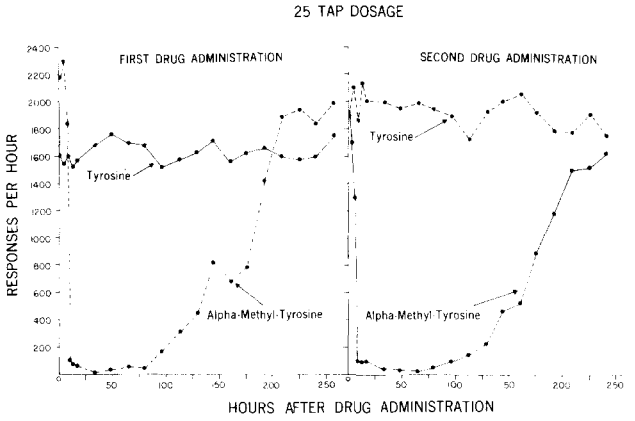

FIG. 2. Mean response rate for rats receiving 25 tap dosage. Solid line represents rats receiving tyrosine first, then AMPT. Broken line represents rats receiving AMPT first, then tyrosine. Drugs were administered at Hour 4 of each graph section (second dot from ordinate). Hour 1 shows mean baseline response rate before each drug administration. Two rats were used in each group.

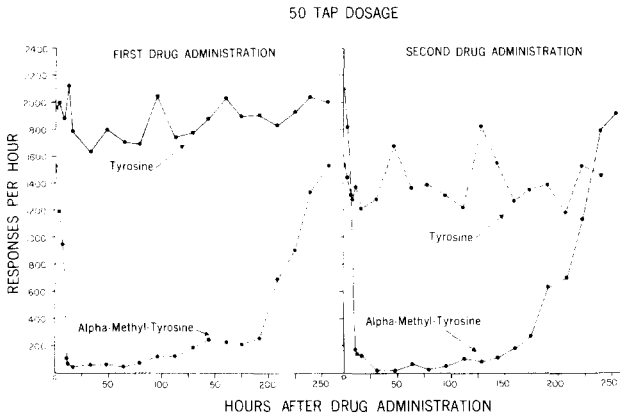

FIG. 3. Mean response rate for rats receiving 50 tap dosage. Solid line represents rats receiving tyrosine first, then AMPT. Broken line represents rats receiving AMPT first, then tyrosine. Drugs were administered at Hour 4 of each graph section (second dot from ordinate). Hour 1 shows mean baseline response rate before each drug administration. Two rats were used in each group.

dosage group reached baseline again between Hours 272–384 (Days 13–17).

Originally, 18 rats were tested in this experiment. However, 2 of the animals stopped bar-pressing immediately after drug administration, one after tyrosine and one after AMPT. After one week of total response suppression, these rats were killed and histology was performed as described earlier. It was found that both of these animals had foreign objects lodged in their brains at the mouth of the chemitrode; apparently, in pushing the inner cannula down through the outer chemitrode, material lodged inside the chemitrode had been pushed into the brain. In one instance the foreign matter was identified as Caulk's Dental Cement, and in the second case the material was unidentifiable. Three other rats continued to bar-press at very high rates for the duration of the first drug test, but upon removing

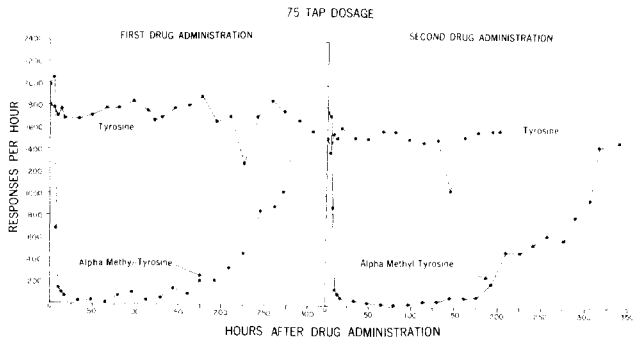

FIG. 4. Mean response rate for rats receiving 75 tap dosage. Solid line represents rats receiving tyrosine first, then AMPT. Broken line represents rats receiving AMPT first, then tyrosine. Drugs were administered at Hour 4 of each graph section (second dot from ordinate). Hour 1 shows mean baseline response rate before each drug administration. Two rats were used in each group.

the inner cannula in order to administer the second drug one week after the first drug administration, it was found that the original drug dosage was still sitting in the tip of the needle, and had not diffused outward into the brain. This may have been due to a build-up of necrotic tissue at the tip of the chemitrode, so that the fluids in the brain could not reach the drug. The cannulae of all other rats showed no traces of drug left upon examination by microscope. One more rat was discarded after the BRS programming unit was inadvertently shorted out across a shock apparatus, and the rat received a long-duration electric shock during Hour 4 of the AMPT drug test. The data from all 6 of these rats were discarded for the purposes of this experiment.

DISCUSSION

Alpha-methyl-para-tyrosine has been shown to cause a decrease in response rate for electrical brain stimulation when administered intraperitoneally, orally, or subcutaneously. It was the purpose of this experiment to determine whether that response rate decrement was due to local inhibition of catecholamine synthesis or to generalized depression effects caused by peripheral administration of the drug. The use of a chemitrode, a device allowing electrical and chemical stimulation of the brain at the same point, made it possible to pinpoint the locus of the drug to within approximately one millimeter of the chemitrode tip [12,13], and thus an exact determination of the structures affected by the chemical was possible. Diffusion into the ventricles was also averted by administration of the drug in crystalline, rather than in solution, form.

The results of this experiment show that AMPT, when administered to the medial forebrain bundle at the dorsal lateral hypothalamus, causes a marked decrease in response rate for ICS. The administration of tyrosine at the same placement causes no change in response rate, indicating that response depression after intracranial AMPT administration is not an artifact of the experimental procedure. These findings are in agreement with other work supporting a monoaminergic system of reward in the medial forebrain bundle of the lateral hypothalamus [3, 4, 5, 18, 22]. These data could also be interpreted as support for the Poschel and

Ninteman theory of a reciprocal interaction between norepinephrine and serotonin in altering reward threshold [19].

It was found that AMPT began to exhibit an effect by Hour 4 after drug administration, and showed major effects between Hour 4 and Hour 8 after administration, results which confirm the findings of previous experiments [3, 18, 20] for the time of onset of response suppression after intraperitoneal AMPT administration. (A more exact determination of the time of the initial response decrement was not possible, since the animals were only tested at 4 hr intervals immediately after drug administration.) This similarity in result can be explained by the fact that peripheral injections take only a few minutes to reach the brain, and once there, drug action parallels that of intracranially administered drugs. However, whereas the above research shows baseline response being resumed within one week after drug administration, the results of this experiment show a significantly longer period of response depression which is directly correlated with the dosage of drug administered intracranially. It seems likely that this greater duration is due to the higher local concentration of AMPT in the neural area in question. Even though dosages of up to 600 mg/kg have been administered intraperitoneally [3,5], much of this quantity does not reach the specific site where stimulation is delivered. However, when minute quantities of 1–5 μg are administered by cannula into the local brain area, the chemical must be assimilated or diffused directly into the neural tissue. While the concentration of tyrosine α-methyl analogue is so much greater than that of the naturally-occurring tyrosine, the kinetics of the rate of reaction will favor the binding of the tyrosine hydroxylase to the AMPT and the subsequent pathway leading to alpha-methyl-dopamine and alpha-methyl-norepinephrine. Therefore, until enough of the AMPT is either broken down enzymatically or otherwise eliminated so that the concentration of tyrosine becomes competitive with the amount of AMPT, very little dopamine or norepinephrine will be synthesized. If the positive nature of the rewarding factor is monoaminergic, the positive reinforcement value of the ICS will then decrease, and response rate should drop to non-stimulation baseline in an extinction curve, as was demonstrated in this experiment.

The results obtained here could be explained equally well biochemically by hypothesizing either a noradrenergic or a dopaminergic system of reward, since AMPT affects the production of both of these putative neurotransmitters. Because much of the dopamine in the brain is contained in the corpus striatum, while the hypothalamus has been shown to be highly adrenergic, it has been postulated that chemically-modified ICS effects elicited from the lateral hypothalamus are due to interference with norepinephrine function [18]. However, recent histochemical fluorescence work in this area has shown large retrograde dopamine build-up in lesioned neurons of the medial forebrain bundle ICS network, with smaller quantities of retrograde norepinephrine, thus implying that dopamine may play a dominant role in the biochemistry of the ICS pathway [4].

It was observed during this experiment that an intracranial dosage of AMPT caused no apparent sedation or loss of activity in rats. All animals appeared to behave normally throughout the 2½ weeks of the experiment, with no decrement in motor response. This suggests that the general sedation observed by other investigators [10, 14, 15, 20, 21] may be due to peripheral effects of the drug, and supports the conclusion of Black and Cooper [3]. These investigators showed parallel data resulting from two separate reward measures after oral AMPT administration, and they postulated that any activity level decrement observed by other experimenters may have been due to a decrease in ICS motivation value only.

The data in the experiment reported in this paper are among the least variable shown in the literature to date on the effects of AMPT on self-stimulation in rats. It was felt that this could be attributed to the very controlled intracranial technique used to deliver the drug, and to the exactness of chemitrode placement, as verified by histology. The results of this experiment lend further support to a theory of monoaminergic reward mediation in the lateral hypothalamus, and suggest that the ICS response suppression shown in the literature after AMPT administration is in fact a function of central inhibition and not a peripheral effect.

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