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Acetylcholine Release in Ventral Tegmental Area by Hypothalamic Self-Stimulation, Eating, and Drinking

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RADA, P. V., G. P. MARK, J. J. YEOMANS AND B. G. HOEBEL. *Acetylcholine release in ventral tegmental area by hypothalamic self-stimulation, eating, and drinking.* PHARMACOL BIOCHEM BEHAV **65**(3) 375–379, 2000.—Evidence is presented for an acetylcholine (ACh) input to the midbrain ventral tegmental area (VTA) as part of a system for self-stimulation and ingestive behavior. Male rats were prepared with an electrode in the perifornical lateral hypothalamus and an ipsilateral guideshaft for microdialysis in the VTA. Extracellular ACh increased in the VTA during self-stimulation, auto-stimulation, eating, or drinking. Infusion of atropine into the VTA via the microdialysis probe was sufficient to stop self-stimulation and reduce intake of food. It is concluded that ACh acts at muscarinic receptors in the VTA as part of a circuit that modulates hypothalamic self-stimulation and ingestive behavior. © 2000 Elsevier Science Inc.

Self-stimulation Eating Drinking Ventral tegmental area Acetylcholine release

THE "reward system" for hypothalamic self-stimulation, eating, drinking, mating and other natural behaviors has been of great interest for 35 years (23). A mesolimbic dopamine component from ventral tegmental area (VTA) to the forebrain has been demonstrated repeatedly (3,5,9,11,24). The present experiment investigates an acetylcholine (ACh) input in the midbrain that excites the mesolimbic dopamine system and, thus, may contribute to the reinforcing functions of the overall system (Fig. 1).

Dopamine cells that project to the NAc arise in the midbrain ventral tegmentum. They receive input from ACh neurons with cell bodies in more posterior parts of the midbrain, specifically the Ch5 cell group localized in the pedunculopontine tegmental nucleus (PPT) and the Ch6 cell group localized in the laterodorsal tegmental nucleus. Some of the ascending ACh axons are thought to make direct contact with dopamine cell bodies in the VTA (25). In this region cholinergic agonists and cholinergic inputs have been shown to stimulate dopamine cells (4,12). These ACh inputs could be important in mood, motivation, or reinforcement by virtue of their influence on the dopamine systems they innervate.

Acetylcholine in the VTA may be necessary for classic self-stimulation behavior. ACh injected into the VTA enhances hypothalamic self-stimulation (18), and cholinergic antagonists can stop it (27,28). As further evidence, inhibition of cholinergic neurons by carbachol (a cholinergic agonist) injected in the Ch5 cell body region attenuates hypothalamic self-stimulation (i.e., raises threshold), whereas scopolamine (a cholinergic antagonist) to block the inhibitory autoreceptors does the opposite (28). These results suggest that ACh released in the VTA might play a key role in hypothalamic self-stimulation. If so, self-stimulation of the lateral hypothalamus (LH) should release ACh. That is what was found in the present study.

If ACh in the VTA is involved in the reinforcement of self-stimulation, then it should play the same role for natural behaviors that normally activate the circuit. Self-stimulation of the hypothalamus and stimulation-induced behavior co-varies with appetites for food or sex, depending on the self-stimula-

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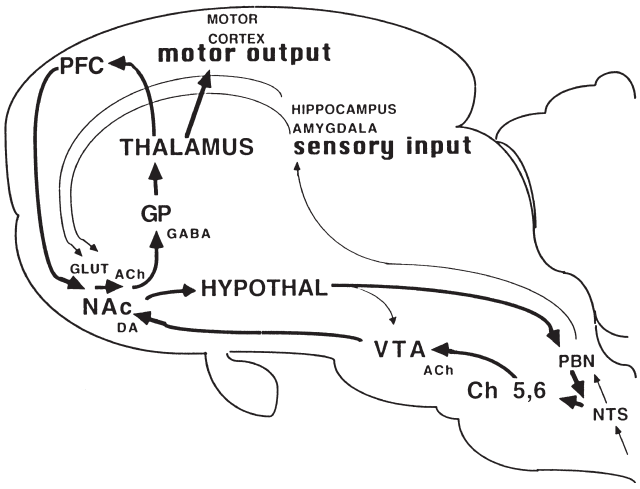

FIG. 1. Schematic side view of the brain to show ACh input to the VTA. This is part of a feeding reinforcement loop from the nucleus accumbens (NAc) to the lateral hypothalamus, which projects to the VTA directly and via the parabrachial nucleus (PBN) and nucleus tractus solitarius (NTS) and then to cholinergic cell groups 5 and 6. Dopamine cells in the VTA project to the NAc. By this route, feeding or self-stimulation controls DA/ACh balance in the NAc to reinforce instrumental behavior. A more detailed version of this figure is given in a recent review (13). Modified from: Hoebel, B. G., et al. Neural systems for reinforcement and inhibition of behavior: Relevance to eating, addiction and depression. In: Kahneman, D., et al., eds. Well being: Foundations of hedonic psychology. Russell Sage Foundation, p560–574, 1999.

tion site (8) and the animal's previous experience (20). When an electrode is placed in the perifornical LH where it will elicit eating, then self-stimulation can be enhanced by food deprivation (1,8,15) and inhibited by obesity or postingestional factors such as stomach distention, a hypertonic gastric load, or calories (10). Thus, self-stimulation can reflect the animal's tendency to eat. If this type of self-stimulation releases ACh in the VTA, then eating should also. And if so, ACh in the VTA might be involved in this natural behavior as well as self-stimulation. There are no studies that report the effect of behaviors such as eating and drinking on the release of endogenous ACh in the VTA. An earlier study did find that injections of cholinergic agonists into the substantia nigra induced feeding, and this was blocked by atropine (22). This could have been due, in part, to nigral projections to the forebrain feeding systems or to diffusion to the nearby VTA.

The present experiment investigates two types of behavior; first self-stimulation, and second, ingestive behavior. In each case brain microdialysis was used to determine if the approach behavior released ACh in the VTA. Then a cholinergic antagonist was infused at the microdialysis site to see if it could block endogenous ACh activation of the rewarded behavior.

METHOD

Animals and Coordinates

Male Sprague–Dawley rats weighing 350–400 g were maintained on a 12 L:12 D reversed light:dark schedule while housed individually with free access to food and water. For stereotaxic surgery, animals were anesthetized with pentobarbital (25 mg/kg, IP) supplemented by ketamine (50 mg/kg, IP). A monopolar platinum-iridium stimulating electrode (0.009 inch) was implanted in the perifornical LH and a stainless steel guide shaft (21 gauge) was secured in the ipsilateral VTA. The following coordinates were used: the LH electrode at: A: −3.0 mm, V: 8.5 mm, L: 1.6 mm; and the VTA guide shaft at A: −6.0 mm, V: 3.6 mm, and L: 0.5 mm with reference to bregma, midsagittal sinus and surface of the leveled skull (17). The microdialysis probe to be inserted later, would extend another 5 mm to reach the VTA. Animals recovered for a minimum of 1 week before experiments (procedures approved by Institutional Animal Care and Use Committee).

Self-Stimulation Training

Rats learned to self-stimulate by lever pressing for a 0.5-s train of monophasic 100-Hz, 0.1-ms square pulses (Tektronix model 160 stimulator) with the output passed through an isolation transformer for zero net current flow in the brain. The pulse intensity was adjusted such that animals would press at a rate of 70 ± 5 responses per min. Animals that would not meet this criterion were excluded from the study. All experiments were carried out during the rats' early dark period.

Microdialysis Technique

Microdialysis probes were constructed of fused silica capillary tubing and 26-gauge stainless steel with a tip of cellulose membrane 1.5 mm long (6,7,14). A probe was lowered into the VTA ipsilateral to the stimulation electrode and cemented to the guide shaft at least 18 h before experimentation. Probes were perfused with a modified Ringer's solution (142 mM NaCl; 3.9 mM KCl; 1.2 mM $CaCl_2$; 1.0 mM $MgCl_2$; 1.35 mM Na_2HPO_4; 0.3 mM NaH_2PO_4, pH 7.3) at a flow rate of 0.5 μl/min overnight and 1 μl/min during the experiment. Neostigmine (0.5 μM) was added to the Ringer as an esterase inhibitor to prevent ACh from degrading.

ACh was measured by reverse phase, high-performance liquid chromatography (HPLC) using an electrochemical detector (E,G & G- Princeton Applied Res., Model 400), dual piston pump (ESA Co. Model 580) and a 20 μl sample loop. ACh was separated on a 10-cm C18 analytical column and then converted to betaine and hydrogen peroxide by an immobilized enzyme reactor (acetylcholinesterase and choline oxidase from Sigma Chem. Co. and columns from Varion Inc.).

Self-Stimulation, Autostimulation, and ACh

The first series of experiments was designed to monitor ACh in the VTA during self-stimulation and then again during automatic stimulation ($n = 5$). Microdialysis samples were collected every 20 min for 1 h before, during, and after LH self-stimulation. ACh was monitored again 8 h and 24 h later while rats received 1 train/s automatic stimulation for 20 min at the prior self-stimulation intensity or half that intensity in counterbalanced order.

To test for a causal relationship between ACh and self-stimulation, atropine was infused into the VTA by reverse dialysis. Atropine (100 μM in the same Ringer's solution) was ipsilaterally perfused through the dialysis probe during self-stimulation on the third day of dialysis. This would reveal whether endogenous ACh in the VTA mediated the self-stimulation response.

Feeding, Drinking, and ACh

In the second experimental series, a different set of animals ($n = 5$) was deprived of food or water for 18 h in the di-

alysis cage. Then 20-min microdialysis samples were collected 60 min before, 20 min during, and 80 min after ingestion of ad lib chow or water. The same procedure was repeated on a second day of dialysis counterbalancing access to food or water. Intake of Purina chow pellets was determined using a piece of paper underneath the cage to recover spillage.

To see if cholinergic action in the VTA is necessary for food intake, the same rats were then left in the dialysis cage overnight with water, but without food, and the next day atropine (100 μM in the probe) was perfused through the probe while food was offered during a 20-min period. This was the third and last possible day of dialysis. The control was an identical test in the same rats without atropine in the Ringer. This was designed to determine whether unilateral cholinergic block in the VTA would reduce meal size.

Statistics and Histology

Microdialyis data were converted to percent of the mean of three baseline samples and analyzed by ANOVA for repeated measures (condition × time). Histology was performed to verify probe and electrode placement in the VTA and LH, respectively. Subjects received an overdose of sodium pentobarbital and were perfused with 0.9% saline followed by formalin. Brains were removed and frozen for sectioning. Sections, 40 microns thick, were taken from anterior to posterior until both probe and electrode tracks were identified. Rats with the dialysis probe outside the VTA area were excluded.

RESULTS

Self-Stimulation or Autostimulation Releases ACh in VTA, and Atropine Blocks Self-Stimulation

As seen in Fig. 2, extracellular ACh in the VTA increased to 198 ± 8% of basal levels during self-stimulation, $F(0, 8) = 38$, $p < 0.001$ ($n = 5$). In successive 20-min intervals, rats lever pressed 1458 ± 147, 1592 ± 139, and 1563 ± 92 times. Figure 2 also shows that during automatic stimulation, ACh again increased to 198 ± 11% of baseline, $F(0, 8) = 31.8$, $p < 0.001$ ($n = 5$). Half-intensity, automatic stimulation had half the effect by increasing ACh to 142 ± 9% of basal levels, $F(0, 8) = 18.38$, $p < 0.001$ ($n = 4$).

Atropine perfused locally through the microdialysis probe completely blocked bar pressing for ipsilateral self-stimulation in three rats and in a fourth decreased it to 58%. The microdialysis probe clogged before the atropine trial in the fifth rat.

Feeding and Drinking Releases ACh in the VTA, and Atropine Reduces Meal Size

Figure 3 shows that during a 20-min meal, ACh in the VTA significantly increased to 167 ± 4.9% of basal levels, returning gradually to initial levels 1 h later, $F(0, 7) = 18.77$, $p < 0.001$ ($n = 5$). Drinking also caused an increase in ACh levels in the VTA to 180 ± 4.8% during the 20-min access to water, followed by a rapid return to basal levels, $F(0, 7) = 13.81$, $p < 0.001$ ($n = 5$).

On a separate day, atropine perfused through the probe unilaterally was sufficient to significantly decrease meal size from 2.8 ± 0.9g to 0.7 ± 0.6g ($T = 2.76$, $p < 0.05$; $n = 5$), as shown in Fig. 4.

DISCUSSION

The first set of experiments shows that LH self-stimulation releases ACh in the VTA. Self-stimulation and auto-stimulation at the same intensity produced the same increase in extracellular ACh levels (Fig. 2). This phenomenon was proportionally reduced from a 98% increase to a 42% increase when the stimulation intensity was reduced to half. Thus, voluntary and automatic stimulation seem to be acting, in part, via the same midbrain ACh pathway. This finding also shows that the rise in extracellular ACh during self-stimulation is not due to pure movement (bar pressing); instead, it argues in favor of ACh in the VTA as playing an important role in some aspect of reward or arousal.

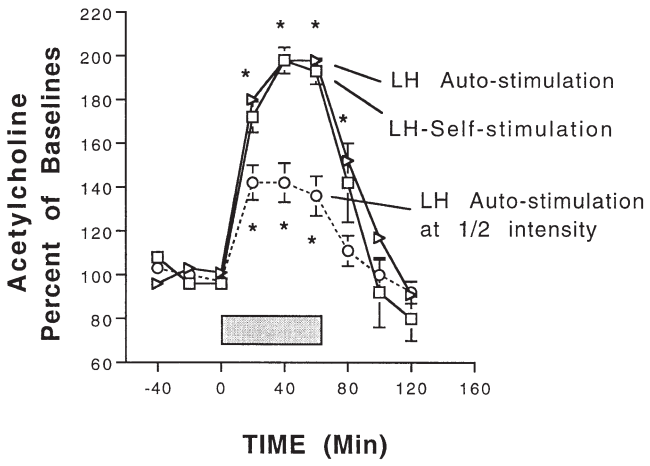

FIG. 2. Self-stimulation or automatic stimulation through an electrode in the perifornical lateral hypothalamus significantly increased extracellular ACh in the ipsilateral VTA measured by microdialysis. Autostimulation at half intensity had half the effect on ACh in the VTA. Horizontal bar indicates hypothalamic stimulation for an hour during three 20-min ACh samples (error bars indicate SEM; Asterisks: $p < 0.01$).

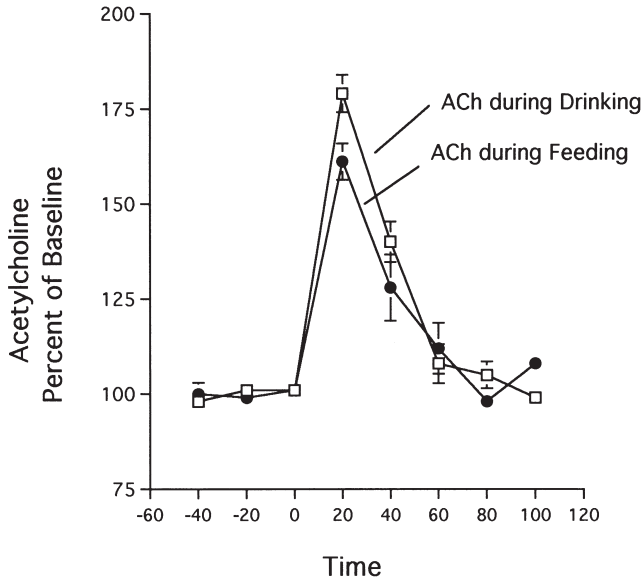

FIG. 3. Deprivation-induced eating or drinking (horizontal bar) for 20 min on different days caused a significant increase in extracellular ACh ($p < 0.001$).

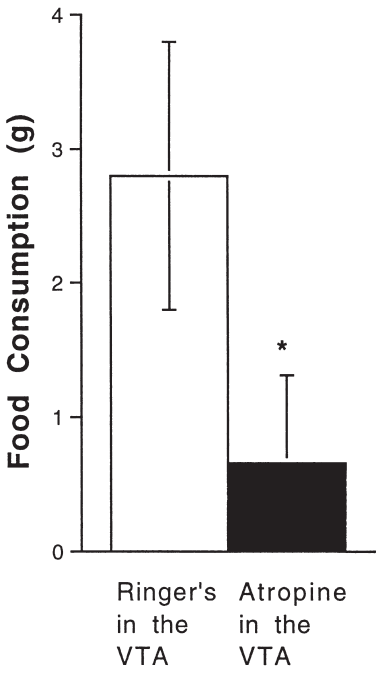

FIG. 4. Atropine added to the microdialysis Ringer totally stopped ipsilateral self-stimulation (not shown) and significantly reduced the size of a 200-min meal (black bar) in food-deprived rats ($p < 0.05$). This suggests that LH self-stimulation and eating act in part via ACh inputs to the VTA.

Self-stimulation was inhibited by a muscarinic antagonist, atropine, infused in the ipsilateral VTA. Atropine is a muscarinic (M1-M5) blocker, so at this time we do not know which of the two receptors is responsible for the attenuation of self-stimulation and ingestive behavior. The atropine probably acted locally in the VTA, not in the hypothalamus, because 3 mm is too far for the drug to diffuse effectively through the brain tissue when infused without pressure. It is more likely that atropine blocked the ACh that is released in the VTA by self-stimulation. This necessary relationship between ACh and self-stimulation confirms in a new way the conclusions from previous studies in which a cholinergic agonist in the VTA increased self-stimulation (i.e., lowered the threshold), and a cholinergic antagonist did the opposite (28). This prior work suggested that muscarinic receptors on the DA cells of the VTA are involved (26). The role of this cholinergic input could be to carry some feature of the reinforcement signal or arousal state or both (19,26).

The self-stimulation circuit that descends from the hypothalamus could go directly and/or indirectly to the VTA. Hypothalamic neurons could project directly to the VTA to stimulate DA cells, including activation of ACh terminals in the VTA for presynaptic release of ACh. This is probably not the main route for releasing ACh, because pharmacological manipulations of the ACh cell body region (Ch5) alter self-stimulation (28). Thus LH self-stimulation probably activates Ch5. The LH electrode could stimulate a relatively direct path to Ch5, or perhaps the circuit goes via other sites such as the parabrachial nucleus (PBN) (2). It is known that LH self-stimulation engages opioid receptors in the parabrachial region that support stimulation-bound feeding and the increase in self-stimulation seen in food deprived rats (1,2). Hypothalamic feeding sites also stimulated sweet taste neurons in the PBN and NTS (16). Therefore, a logical possibility is that the PBN and NTS sends this information to Ch5, and hence, to the VTA (Fig. 1).

In the second experiment, a significant increase in ACh release was observed when the animals ate food or drank water. Ingestion of food was partially inhibited by unilateral atropine infusion through the dialysis probe. Atropine completely abolished self-stimulation. This differential effect could be explained by the existence of a more complex brain circuit involved in the control of feeding. It could also simply mean that unilateral atropine was capable of completely blocking a unilateral electrical self-stimulation pulse. It might be necessary to infuse the VTA bilaterally with atropine to completely block feeding. These results suggest that ACh in the VTA is involved in conveying the reinforcement or arousal value not only for self-stimulation, but also for natural behaviors such as feeding and drinking.

Others have shown that infusion of ACh agonists into the VTA can increase extracellular dopamine in the nucleus accumbens (21). Therefore, the ACh release demonstrated by the present experiments is probably one means by which self-stimulation and ingestive behavior release DA in the NAc and other forebrain sites. In conclusion, an ACh input to the VTA at muscarinic receptors is necessary for self-stimulation and feeding. Midbrain cholinergic cells may help stimulate DA cells as a component of the circuitry for feeding reinforcement.

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