Dopaminergic Synapses Mediate Neuronal Changes in an Analogue of Operant Conditioning

R. NARGEOT, D. A. BAXTER, G. W. PATTERSON, AND J. H. BYRNE
Department of Neurobiology and Anatomy and W.M. Keck Center for Neurobiology of Learning and Memory, The University of Texas-Houston Medical School, Houston, Texas 77030

Nargeot, R., D. A. Baxter, G. W. Patterson, and J. H. Byrne. Dopaminergic synapses mediate neuronal changes in an analogue of operant conditioning. J. Neurophysiol. 81: 1983–1987, 1999. Feeding behavior in Aplysia can be modified by operant conditioning in which contingent reinforcement is conveyed by the esophageal nerve (E n.). A neuronal analogue of this conditioning in the isolated buccal ganglia was developed by using stimulation of E n. as a analogue of contingent reinforcement. Previous studies indicated that E n. may release dopamine. We used a dopamine antagonist (methylergonovine) to investigate whether dopamine mediated the enhancement of motor patterns in the analogue of operant conditioning. Methylergonovine blocked synaptic connections from the reinforcement pathway and the contingent-dependent enhancement of the reinforced pattern. These results suggest that dopamine mediates at least part of the neuronal modifications induced by contingent reinforcement.

INTRODUCTION

Dopamine appears to play a critical role in several examples of learning, including operant conditioning. For example, injection of dopamine antagonists or destruction of dopamine neurons produces deficits in operant conditioning. Moreover, dopaminergic neurons are activated by reinforcing stimuli, and electrical or pharmacological stimulation of dopaminergic systems in the brain can mediate reinforcement (for recent reviews see Ettenberg 1989; Le Moal and Simon 1991; Schultz 1997, 1998). Despite the involvement of dopamine in reinforcement, little is known about the cellular mechanisms underlying its effects.

In recent studies we have begun to investigate the cellular changes induced by contingent reinforcement in an analogue of operant conditioning of feeding in the isolated buccal ganglia of Aplysia (Nargeot et al. 1997, 1999a,b). Dopamine is the primary transmitter believed to be released by the reinforcement pathway (Kabotyanski et al. 1998a). Thus in this study we tested whether methylergonovine, which primarily affects dopaminergic transmission (Ascher 1972; Buckett et al. 1990; Drummond et al. 1980; Swann et al. 1978; Teyke et al. 1993; Wright and Walker 1984), could prevent the effects of contingent stimulation of the reinforcement pathway. A preliminary report of these results appeared in abstract form (Baxter et al. 1998).

METHODS

The experimental procedures of this study were similar to those described previously (Nargeot et al. 1997). Buccal ganglia were isolated and pinned out in a Sylgard-coated petri dish containing either control saline [i.e., artificial seawater (ASW)] alone or ASW containing methylergonovine. Methylergonovine was the only antagonist examined in this study. The solutions were maintained at 15°C in a static bath by means of a Peltier cooling device. Normal ASW was composed of (in mM) 450 NaCl, 10 KCl, 30 MgCl2 (6 H2O), 20 MgSO4, 10 CaCl2(2H2O), and 10 Trizma, pH adjusted to 7.4. In some experiments, a solution of ASW containing high concentrations of Ca2+ and Mg2+ (165 mM MgCl2 and 30 mM CaCl2) was used to block polysynaptic pathways (Byrne et al. 1978). Solutions of methylergonovine (Sigma Chemical, St. Louis, MO) were prepared in normal or modified ASW immediately before the experiments. The experimenter was not aware of either the type of the solution (ASW or methylergonovine) or the concentration (0.1 nM to 1 μM) that was used. Preparations were bathed in the solutions for ≥30 min before recordings were made.

Conventional extracellular and intracellular nerve stimulation and recording techniques were used. Rhythmic motor activity was elicited by monotonic (2 Hz) stimulation of an afferent nerve (n.2,3) (for details see Nargeot et al. 1997). This rhythmic activity was composed of different motor patterns (i.e., pattern I, pattern II, and intermediate patterns; see Fig. 1). The paradigm for stimulating the reinforcement pathway (esophageal nerve, E n.2) in the analogue of operant conditioning was described by Nargeot et al. (1997). In the contingent reinforcement group, beginning with the first occurrence of pattern I, stimulation of E n.2 was contingent on occurrences of this pattern. Training continued for 10 min, and a minimum of five stimulations of E n.2 was required. In the yoke-control group, the stimulation of E n.2 was delivered with the same timing and parameters as in a paired contingent-reinforcement preparation. The monotonous stimulation of n.2,3 was stopped at the end of the training period and restarted for 20 min, beginning 60 min after training. Data were collected during the last 10 min of this stimulation period.

RESULTS

High concentrations of methylergonovine blocked rhythmic motor patterns

In the isolated buccal ganglia, rhythmic motor patterns (i.e., pattern I, pattern II, and intermediate patterns), which were similar to those observed during consummatory feeding behaviors (e.g., ingestion and egestion), were induced by monotonic stimulation of n.2,3 (Fig. 1A). Pattern I was similar to the motor pattern observed during ingestion in freely behaving animals, and pattern II was similar to motor pattern observed during egestion (Morton and Chiel 1993). We first examined...
Methylergonovine blocked monosynaptic effects of the reinforcement pathway

Although 1 nM of methylergonovine had very little effect on the rhythmic activity induced by stimulation of n.2,3, we tested whether this concentration affected synaptic connections from the reinforcement pathway (i.e., E n.2) to three identified cells in the buccal ganglia (i.e., B4/5, B51, and B52). These cells are believed to be part of the central pattern generator (CPG)-mediating aspects of feeding (Fig. 2). For example, we found that E n.2 made an apparent monosynaptic connection with B51, a neuron whose properties were modified by contingent reinforcement (Nargeot et al. 1999a). This connection persisted in solutions that contained high concentrations of divalent ions and had a fast inhibitory component that was elicited by a single stimulus (0.5 ms) to E n.2 (Fig. 2) and a slow excitatory component that was elicited by high-frequency stimulation of E n.2 (i.e., 10 Hz, 6 s; not shown). A concentration of 1 nM of methylergonovine reduced both the fast inhibitory (Fig. 2A) and the slow excitatory components (not shown) of the E n.2-mediated synaptic potential (n = 5 preparations). We did not examine a full range of concentrations, but a high concen-

FIG. 1. Dose–inhibition relationship of methylergonovine on rhythmic motor patterns. A–C: rhythmic buccal motor patterns recorded in the nerve to the intrinsic muscle 2 (I2 n.) and the radial nerve (R n.) were induced in artificial seawater (control, A) by monotonic (2 Hz) stimulation of n.2,3. Different patterns were distinguished by the phase relationship of the closure activity (large amplitude activity in R n.) relative to the protraction phase (activity in I2 n.). In pattern I (solid circles), ≈50% of the total closure activity expressed during the pattern occurs after termination of the protraction phase (vertical dashed line). In the other patterns (open circles) the closure activity occurs only during the protraction phase (in pattern II), or >50% of the closure activity occurs during protraction phase (in intermediate patterns). The frequency of the rhythmic activity was reduced in solutions containing 10 nM (D) or 1 μM of methylergonovine (C). D: dose–inhibition relationship of methylergonovine on rhythmic motor patterns. The number of patterns induced during a 20-min period of monotonic stimulation of n.2,3 was normalized to the mean number of patterns observed in control saline. Separated groups of preparations were used for each concentration of methylergonovine (n indicated in brackets) and for control saline (n = 6). Data were fitted by a sigmoid curve (e.g., Santana et al. 1998): \( y = a + (1 - a)/(1 + x/K) \) with a = 0.22 and K = 8.4 nM.

the effect of methylergonovine on the rhythmic motor program induced by stimulation of n.2,3.

The frequency of occurrences of motor patterns decreased with increasing concentrations of methylergonovine (Fig. 1, B–D). A dose–inhibition relationship of the effect of methylergonovine on the rhythmic motor pattern indicated that the apparent concentration of methylergonovine that induced a one-half inhibitory effect (IC50) was 8.4 nM (Fig. 1D).

![Diagram of the apparent monosynaptic connection from E n.2 to B51](http://jn.physiology.org/)

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tration (1 μM) of methylergonovine abolished these E n.2-mediated synaptic potentials (Fig. 2R; n = 2 preparations).

Synaptic connections from E n.2 that persisted in solutions containing high concentrations of divalent ion were also observed in cells B4/5 and B52 (Fig. 2C). Methylergonovine (1 μM) abolished the E n.2-mediated synaptic potentials in B4/5 (n = 3 preparations) and B52 (n = 2 preparations). We did not examine the effects of lower concentrations of methylergonovine on these synaptic connections. In all cells examined (i.e., B4/5, B51, and B52) the effects of methylergonovine (1 μM) were only partially reversed by prolonged washing (>1 h) with control saline. B64 is another CPG neuron that received apparent monosynaptic input from E n.2, but the effect of methylergonovine on the E n.2-mediated synaptic potential in this cell was not examined.

A previous study (Wright and Walker 1984) found that in some molluscan neurons ergonovine could block serotonin (5-HT)-induced hyperpolarizations but not 5-HT-induced depolarizations. To determine whether the E n.2-mediated hyperpolarization of B51 may be mediated via 5-HT, we investigated whether exogenous application of 5-HT mimicked the actions of the E n.2 and hyperpolarized B51. In three preparations bath application of 5-HT (5 μM) induced only a slight depolarization (1 ± 1 mV; values are means ± SE) of the resting membrane potential of B51. In contrast, bath application of dopamine mimicked aspects of E n.2 stimulation and hyperpolarized B51 (Kabotynski et al. 1998b). The results indicated that 5-HT is unlikely to mediate the inhibitory actions of E n.2 on B51 and that low concentrations of methylergonovine affect the efficiency of the reinforcement pathway. Moreover, these results suggested that dopamine may be part of the reinforcing processes.

Methylergonovine blocked contingent-dependent enhancement of motor patterns

To investigate whether methylergonovine modified the effect of contingent reinforcement in an analogue of operant conditioning, we used four groups of preparations (Fig. 3). Two groups were bathed in 1 nM methylergonovine (Fig. 3B). In a contingent-reinforcement group, stimulation of E n.2 was contingent on pattern I during the training period (for details see Nargeot et al. 1997). In a yoke-control group, timing of the stimulation of E n.2 was determined by a paired contingent-reinforcement preparation, and there was no contingency with the ongoing patterns in the yoke-control preparation. Two additional groups received the same stimulation paradigms (contingent reinforcement and yoke control) but were bathed in control saline (Fig. 3A). The number of occurrences of pattern I (i.e., the reinforced pattern) during a 10-min test period beginning ~1 h after training was compared between groups.

Statistical comparisons (i.e., two-way analysis of variance) indicated a significant difference in the effects of the training paradigms (F = 4.671, df = 40, P < 0.05) that depended on the type of solution used (F = 4.671, df = 40, P < 0.05) (Fig. 3). Post hoc pairwise comparisons indicated that in control saline the number of occurrences of pattern I was significantly enhanced in the contingent-reinforcement group compared with the yoke-control group (q2 = 4.322, P < 0.005; Fig. 3A). In contrast, in the presence of methylergonovine contingent reinforcement did not increase the occurrences of pattern I (Fig. 3B). The contingent-reinforcement paradigm also significantly enhanced the number of occurrences of pattern I in control saline compared with that in methylergonovine (q2 = 4.171, P < 0.01; Fig. 3). Moreover, as expected, in the absence of contingent reinforcement and because of the apparent absence of effect of 1 nM methylergonovine on the ongoing rhythmic activity, no significant change was observed between yoke-control groups in either solutions or in the number of occurrences of the nonreinforced patterns (e.g., pattern II and intermediate patterns) of the four different groups (F = 0.008, df = 40).

DISCUSSION

The results indicated that methylergonovine has at least three effects in the buccal ganglia of Aplysia. First, sufficiently high concentrations of methylergonovine (1 μM) disrupted rhythmic buccal motor patterns induced by monotonic stimulation of n.2,3. Second, a low concentration of methylergonovine (1 μM) reduced E n.2-mediated synaptic potentials, whereas a higher concentration (1 μM) blocked synaptic connections from this reinforcement pathway. Third, a lower concentration of methylergonovine (1 nM) blocked the enhancement of motor patterns induced by contingent stimulation of E n.2 in an analogue of operant conditioning.

In various gastropod mollusks, ergot alkaloids and their derivatives have been shown to have several different effects, including inhibiting the binding of dopamine and to a lesser extent 5-HT to receptors (Drummond et al. 1980); acting as a dopamine agonist and partial agonist (Gospe and Wilson 1981; Ku and Takeuchi 1983, 1986; Miyamoto et al. 1979, 1980); and acting as dopamine antagonist and mixed antagonist
(Buckett et al. 1990; Gospe and Wilson 1981; Juel 1983; Lukyanetz and Kostyuk 1996; Pasic et al. 1987; Wright and Walker 1984). Some reports indicate that these agents have no effect on dopaminergic transmission (Magoski et al. 1995) or dopamine-stimulated adenylyl cyclase activity (Deterre et al. 1986; Yamane and Gelperin 1987). Although the pharmacological actions of methylergonovine were not characterized extensively in *Aplysia*, methylergonovine is believed to act as a dopamine antagonist at some dopamine receptors (Ascher 1972; Gospe and Wilson 1981; Swann et al. 1978; Teyke et al. 1993). This study did not examine the actions of methylergonovine on exogenous dopamine, but the results did illustrate that methylergonovine blocked the synaptic inputs from a neural pathway (i.e., E.n.) previously shown to stain positively for dopamine (Kabotyanski et al. 1998a). Thus our results suggest that dopamine plays an important role in both the genesis of feeding behavior and learning-induced changes in this behavior.

Several lines of evidence support the conclusion that dopamine plays an important role in the genesis of feeding behavior in *Aplysia*. First, several putative dopaminergic cells were characterized in the neural circuitry that mediates feeding in *Aplysia* (Kabotyanski et al. 1998a; Rosen et al. 1991; Teyke et al. 1993). These cells express rhythmic activity during fictive feeding. Second, dopaminergic cells were found to participate in the CPG in isolated buccal ganglia, and direct depolarization of these dopaminergic cells can drive buccal motor patterns that were associated with feeding (Kabotyanski et al. 1998a; Teyke et al. 1993). Third, application of exogenous dopamine (or its metabolic precursor) induces feeding-like movements in *Aplysia* (Kabotyanski et al. 1998a; Kabotyanski et al. 1998b). Characterization of motor programs generated in isolated buccal ganglia (Baxter et al. 1995). Thus, together with the observation that methylergonovine blocked rhythmic motor patterns in the buccal ganglia, these results indicate that dopamine is one of the key transmitters used by the feeding circuit in *Aplysia*. Similar roles for dopamine in feeding were suggested in other invertebrates and vertebrates (Berry and Cottrell 1973; Evans and Eikelboom 1987; Kemenes 1997; Kemenes et al. 1990; Kyriakides and McCrohan 1989; Martel and Fantino 1996; Orosco et al. 1995; Sweeney 1963; Terry et al. 1995; Weiland and Gelperin 1983; Wise and Raptis 1986; Zhou and Palmiter 1995).

In addition to playing a role in the genesis of rhythmic activity, this study suggests a second role for dopamine in feeding. Specifically, it appears to mediate the reinforcement during operant conditioning. Several lines of evidence support this conclusion. First, the results of histofluorescent studies suggest that dopamine is the primary neurotransmitter in the reinforcement pathway (Kabotyanski et al. 1998a). Second, apparent monosynaptic effects from the reinforcement pathway were affected by a dopamine antagonist. Third, this antagonist suppressed the enhancement of motor patterns induced by contingent reinforcement. This latter effect is unlikely to result from an action of methylergonovine on the genesis of the rhythmic motor activity because this activity was not significantly different in control saline and methylergonovine in the yoke-control groups (Fig. 3). Moreover, no modification was observed for the number of nonreinforced patterns in the different groups. Finally, the dose-inhibition relationship of methylergonovine on the rhythmic activity indicated that 1 nM of methylergonovine, which was used to block the contingent-dependent enhancement, had virtually no effect on the genesis of the rhythmic activity (e.g., Fig. 1). The different sensitivity to methylergonovine of the dopamine-dependent processes that mediate the genesis of rhythmic activity and reinforcement suggests that different types of dopamine receptors are involved. An important goal for future research will be to identify and characterize the presumptive dopamine-containing neurons in the reinforcement pathway and examine the mechanisms by which dopamine exerts its effects on postsynaptic target cells such as B51.

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Address for reprint requests: J. H. Byrne, Dept. of Neurobiology and Anatomy, W. M. Keck Center for Neurobiology of Learning and Memory, The University of Texas-Houston Medical School, P. O. Box 20708, Houston, TX 77225.

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REFERENCES


