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

INTRODUCTION

Dopamine appears to play a critical role in several examples of learning, including operant conditioning. For example, inhibition 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 MgCl$_2$ (6H$_2$O), 20 MgSO$_4$, 10 CaCl$_2$(2H$_2$O), and 10 Trizma, pH adjusted to 7.4. In some experiments, a solution of ASW containing high concentrations of Ca$^{2+}$ and Mg$^{2+}$ (165 mM MgCl$_2$ and 30 mM CaCl$_2$) 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 mM) 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 monotonic 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-

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 (IC$_{50}$) was 8.4 nM (Fig. 1D).

![Diagram of the apparent monosynaptic excitatory (○ and ▲) and inhibitory (○ and ▼) connections from E n.2 to 4 identified neurons of the central pattern generator. ▲ and ▼: fast (~ 40–120 ms) synaptic effects elicited by a single (0.5 ms) shock to E n.2. ◦: slow (>500 ms) synaptic effects of the stimulation of E n.2. In B51, the slow synaptic component was elicited by a high-frequency stimulation (10 Hz, 6 s). In cells B4/5, B52, and B64, slow synaptic components were elicited by a single (0.5 ms) shock of E n.2. ▲: synapses that were blocked with 1 µM of methylergonovine (B2) and was partially reversed after washout (B3). Four oscilloscope traces that were recorded during the steady state are superimposed in each case. Membrane potential of B51 was held at −40 mV. A: single (0.5 ms; arrow) stimulation of reinforcement pathway (i.e., E n.2) elicited an inhibitory postsynaptic potential in neuron B51 in a control solution that was rich in divalent ions (A1; see also B1). The strength of this synapse was reduced by addition of 1 nM of methylergonovine (A2) and was partially reversed after washout (A3). During successive stimulation the amplitude of the inhibitory postsynaptic potential decreased rapidly before reaching a steady state. Four oscilloscope traces that were recorded during the steady state are superimposed in each case. Membrane potential of B51 was held at −40 mV. C: summary diagram of the apparent monosynaptic excitatory (○ and ▲) and inhibitory (○ and ▼) connections from E n.2 to 4 identified neurons of the central pattern generator, ▲ and ▼: fast (~ 40–120 ms) synaptic effects elicited by a single (0.5 ms) shock to E n.2. ◦: slow (>500 ms) synaptic effects of the stimulation of E n.2. In B51, the slow synaptic component was elicited by a high-frequency stimulation (10 Hz, 6 s). In cells B4/5, B52, and B64, slow synaptic components were elicited by a single (0.5 ms) shock of E n.2. ▲: synapses that were weakened by application of 10 $\mu$M methylergonovine. The effect of methylergonovine on the E n.2-mediated synaptic potentials in B64 was not tested.
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 (Kabotyanski 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 nM) 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.
of methylergonovine, which was used to block the contingent-observed for the number of nonreinforced patterns in the dif-
cyke-control groups (Fig. 3). Moreover, no modification was
cantly different in control saline and methylergonovine in the
rhythmic motor activity because this activity was not signifi-
cate from an action of methylergonovine on the genesis of the
contingent reinforcement. This latter effect is unlikely to result
were affected by a dopamine antagonist. Third, this antagonist
reinforcement pathway (Kabotyanski et al. 1998a). Second,
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 dopa-
mine 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
semi-intact preparations (Kabotyanski et al. 1995) and feeding-
related motor patterns in isolated buccal ganglia (Baxter et al.
1995). Thus, together with the observation that methylergon-
ioine 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 Gottrell 1973; Evans and Eikelloo
Sweeney 1963; Terry et al. 1995; Weiland and Gelperin 1983;

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 signifi-
cantly 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 dif-
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 in-
volved. An important goal for future research will be to iden-
tify and characterize the presumptive dopamine-containing neurons in the reinforcement pathway and examine the mecha-
isms by which dopamine exerts its effects on postsynaptic
target cells such as B51.

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Present address of R. Nargeot: Université Bordeaux I, Laboratoire de Neurobiologie des Réseaux, Bât. Biologie Animal-Bz, Avenue de Facultés, 33405 Talence Cedex, France.

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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DOPAMINE-MEDIATED REINFORCEMENT

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