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

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 an 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). 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(6H₂O), 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 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 (IC50) was 8.4 nM (Fig. 1D).
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 \( q_{52} = 4.322, P < 0.005; \) 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 \( q_{52} = 4.171, P < 0.01; \) 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.
ters used by the feeding circuit in these results indicate that dopamine is one of the key transmitters 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.

We thank F. D. Lorenzetti for helping us examine the effects of serotonin on the resting membrane potential of cell B51.

This research was supported by the Ernst Knobil Fellowship, Grant 011618-048 from the Texas Higher Education Coordinating Board, and National Institute of Mental Health Grant MH-58321 and Award K05 MH-00649.

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.

Received 3 August 1998; accepted in final form 10 December 1998.

REFERENCES


LUKYNENZ, E. A. AND KOSTYUK, P. G. Two distinct receptors operate the cAMP cascade to up-regulate L-type Ca channels. Pflügers Arch. 432: 174–181, 1996.


