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Dopamine modulates synaptic activity in the optic lobes of cuttlefish, *Sepia officinalis*

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Abstract

The effects of dopamine on spontaneous excitatory postsynaptic currents (sEPSCs) and inhibitory postsynaptic currents (sIPSCs) in three different classes of neurones within the optic lobe of cuttlefish were investigated using whole-cell voltage clamp techniques in a slice preparation. The neuronal types were centrifugal and amacrine neurones, located in the inner granular cell layer, and medullar interneurones, located within the central medulla of the optic lobes. The results demonstrate that bath application of dopamine (50 μM) reversibly reduced both the frequency and amplitude of sEPSCs and of sIPSCs in these optic lobe neurones. The inhibitory effects of DA were dose-dependent and neither D_1- nor D_2-like receptors appear to be implicated, but probably D_4-like receptors are involved in these actions. By pre-applying tetrodotoxin (TTX, 0.5 μM), to block action potential-dependent EPSCs and IPSCs, it is shown that dopamine has no effect on the amplitude, frequency or decay time constant of the mEPSCs or mIPSCs. The results are the first to identify a specific physiological action of dopamine on cephalopod brain activity, they indicate that this effect is probably presynaptic to the specific classes of cells recorded from, and they provide information on the pharmacological profile of the receptors involved. The widespread inhibitory effect of dopamine on the activity of cuttlefish optic lobe neurones is discussed in the context of comparable data from vertebrate preparations and the actions of other neuromodulators in the cuttlefish brain.

Keywords: Cephalopod; Voltage-clamp; mEPSCs; mIPSCs

Cephalopods, such as octopus, squid and cuttlefish, have some of the largest and most complex nervous systems amongst the invertebrates with processing and computational capabilities that rival those of vertebrates [22,29]. In particular, the cephalopod visual system has performance characteristics comparable with those of analogous vertebrate systems and, like these, localises the central processing of visual information within distinct areas of the brain; in cephalopods, these areas are the largest lobes in the brain, the optic lobes [22]. In cuttlefish, the optic lobes are bean-shaped structures lying just behind each eye and comprising an outer, regularly arranged cortex, also called the "deep retina" by Cajal [6], and an inner central medulla [32]. As well as processing the afferent projections from the retina, the optic lobes also contain part of the visual memory store and operate as an intermediate motor centre for control of the skin colour, through the chromatophore system [7,31]. Although considerable morphological information is available on the cellular structure of the optic lobes [5,32], little is known of the underlying physiological activity (e.g. [30]). Nevertheless, a number of biochemical and immunohistochemical studies (reviewed [20]) have identified the main neurotransmitters and neuromodulators present in cephalopod brains and shown that dopamine (DA), although localised in a number of brain regions, has its highest concentration, and is the most prevalent transmitter substance, in the optic lobes [16].

DA is already known to be a major modulatory neurotransmitter with a widespread distribution within the vertebrate CNS [15] and can act at postsynaptic, presynaptic and extrasynaptic sites [25,28]. Opposing influences of D_1 and D_2 receptor activation on cAMP-dependent signaling have been

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reported in many studies (reviewed, [21]), with D_1 receptors acting through the stimulatory G_s-like G_{olf}, and D_2 receptors acting through the inhibitory $G_{i/o}$ proteins.

The present work uses a cephalopod brain slice preparation to determine whether DA is physiologically active within the brain, to describe DA's effects on the activity of three classes of identified cell types and provide an initial description of the pharmacological profile of the receptors involved. The recently developed optic lobe preparation [8] is employed here because this region has been reported to have the highest DA concentrations in the brain [16]. Furthermore, DA has already been histochemically localised in a specific class of optic lobe neurones, the centrifugal cells [20], these cells are efferent neurones projecting to the retina where DA has been shown to influence both the responses of the photoreceptors to light [27,18] and the migration of the retinal screening pigment [14].

Cuttlefish, *Sepia officinalis*, of both sexes were used in this study. For experiment, an animal was anesthetized in 2% ethanol, killed by decapitation and the optic lobe slices prepared as previously described [8]. The cortex region of the slice is composed of layers of outer and inner granular cells separated by a dense neuropil region called the plexiform zone [32]. The central medulla region of the optic lobe slice is less well structured but contains numerous clusters of cells, the cell islands, separated by neuronal tracts and neuropil areas [32]. For electrophysiological recordings, individual neurones were identified visually using an Olympus BX50WI upright microscope, with a ×40 water immersion objective lens, and equipped with an infrared illumination and a video enhanced visualization system (Hamamatsu Photonics, Ltd., Hertfordshire, UK) consisting of a CCD camera (C7500) and its controller (C2741-90). Individual slices were transferred to the recording chamber where they were fully submerged and superfused with oxygenated artificial seawater (ASW) at a rate 2–3 ml/min. The composition of the ASW was as follows (in mM): 430 NaCl; 10 KCl; 10 $CaCl_2$; 30 $MgCl_2$; 25 $MgSO_4$; 0.5 KH_2PO_4; 2.5 $NaHCO_3$; 10 glucose; 10 HEPES. pH was adjusted to 7.8 with NaOH. The osmolarity of this external solution was around 997 Osm mol/kg^{-1} H_2O. For whole cell patch clamp [10], pipettes were pulled from soda glass capillaries (Intracel, 1.5 mm o.d. × 0.86 mm i.d.) and had tip resistances typically between 2 and 4 MΩ when filled with a potassium gluconate-based internal solution. Recordings were made using an Axopatch amplifier (200A, Axon Instruments, Union City, USA) controlled by PClamp8 software (Axon Instruments) for data collection and storage. The data acquisition sampling rate was set to 10 kHz and the signals were low-pass filtered at 2 kHz. The pipette series resistance was electronically compensated, as far as possible, to give voltage errors of only few mV at peak current levels. The capacitance current response to a −10 mV voltage step, from a holding potential of −60 mV, was typically recorded for each neurone and the access resistance calculated. Liquid junction potential was estimated using the liquid junction calculator provided by Axon Instruments and incorporated into the calculations of reversal potentials for both sEPSCs and sIPSCs. Previous direct measurements of liquid junction potentials have validated this methodology. The internal solution for recording synaptic currents contained (in mM): 500 K-gluconate; 10 NaCl; 4 $MgCl_2$; 3 EGTA; 20 HEPES, 2 Na_2ATP, 0.2 Na_3GTP, 0.2 Lucifer Yellow CH (lithium salt). pH and osmolarity were adjusted to 7.4 and 870 Osm mol kg^{-1} H_2O with KOH and sucrose, respectively.

Dopamine (DA) (Sigma Chemicals) was prepared just before use and was bath applied approximately 10–15 min after achieving whole-cell membrane seal and breakthrough. Sodium metabisulfite ($Na_2S_2O_5$, 50 μM) was used as an antioxidant to protect DA in solution [33]. Control recordings from five optic lobe neurones demonstrated that the bath application of $Na_2S_2O_5$ at concentrations ranging from 10 to 100 μM had no effect on synaptic transmission in the optic lobe slice preparation. Although acknowledging that cuttlefish DA receptors may not map precisely onto the receptor subtypes found in vertebrates [11], a selection of DA receptor agonists (SKF 38393; Quinpirole) and antagonists (sulriride, haloperidol) were tested for their effects. TTX (Tocris), at a concentration of 0.5 μM, was added to the external bathing solution in order to block action potential-dependent synaptic transmission, thus permitting the investigation of whether or not dopamine had any effect on mEPSCs and mIPSCs [1].

The recordings of sEPSCs and sIPSCs activity were analyzed using the MiniAnalysis software (Synaptosoft Inc., Decatur, USA) to provide measurements of PSC amplitude, frequency and decay time constants. Where statistical comparisons are made before and after the application of dopamine, a two-tailed paired Student's *t* test is employed. If not stated otherwise, data were denoted as statistically significant when $p < 0.05$.

Whole-cell patch clamp recordings were obtained from representatives of three separate classes of optic lobe neurones; these were (1) centrifugal cells, with cell bodies in the inner granular cell layer and axons which pass across the plexiform zone and exit the optic lobe in the optic nerves as efferents to the retina, (2) small amacrine neurones which lie on the border between the inner granular cell layer and in the neuropil of the plexiform zone and are local interneurones, and (3) medullar neurones lying within the cell islands of the central medulla region and have local connections to neighbouring islands. Spontaneous excitatory postsynaptic currents (sEPSCs) were invariably detected in recordings from all three of these neuronal types. Spontaneous inhibitory postsynaptic currents (sIPSCs) were observed mainly in medullar and amacrine neurones and only rarely in centrifugal neurones (∼7%). The medullar and amacrine neurones are known to receive glutamatergic excitatory inputs and GABAergic inhibitory synaptic inputs [8], whereas the centrifugal cells of the inner granular cell layer receive predominantly excitatory synaptic inputs, but these can be cholinergic as well as glutamatergic [8,9].