candidate than somatostatin as a satiety factor, since PP is detected in the systemic circulation[15,17,23] (whereas such may not be the case for somatostatin) and since the plasma concentration of PP indeed increases in response to food intake[15–18].

The present work reveals that exogenous PP, when administered to the hyperphagic ob/ob mice, reduces food intake and causes a dose-related decrease in body weight gain. These results are compatible with the hypothesis that PP participates in the regulation of food intake. The demonstration that hyperphagia in obese mice is indeed associated to decreased levels of circulating pancreatic polypeptide awaits the availability of a method of measurement of PP in murine plasma.

Adenosine 3′,5′-monophosphate in snail (Helix pomatia) nervous system: Analysis of dopamine receptors

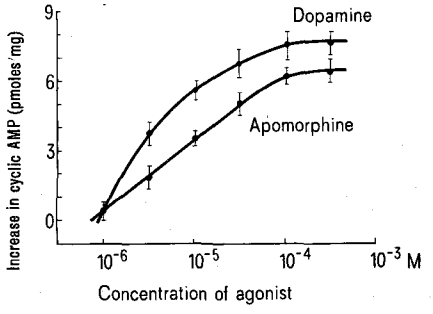
N. N. Osborne[1]

Max-Planck-Institut für Experimentelle Medizin, Forschungsstelle Neurochemie, D–3400 Göttingen (Federal Republic of Germany, BRD), 24 September 1976

Summary. The effect of dopamine on snail (Helix pomatia) nervous tissue adenosine 3′, 5′-monophosphate content was examined. The results show support for the idea that the dopamine receptors in the snail nervous system involve adenylate cyclase. It is suggested that these are the dopamine excitatory receptors rather than the inhibitory ones.

Considerable evidence has accumulated implicating dopamine as a neurotransmitter in the snail brain. It is localized in a small proportion of neuron perikarya, their axons and presumed presynaptic endings[2–4], and all the data from experiments on the release of dopamine from a single neuron containing the amine[5], the occurrence of dopamine receptors on certain gastropod neurons[5–7], the presence of dopamine within synaptic-type vesicles[8] and the demonstration of a high affinity uptake process for dopamine[9] tend to substantiate the hypothesis that the amine is involved in some specific aspect of function in the snail CNS. With regard to the nature of the dopamine receptor in the gastropods, a number of electrophysiological studies have been carried out suggesting that at least 2, and probably more, types of dopamine receptor exist in their nervous tissues[10]. Moreover, an adenylate cyclase, which is activated by low concentrations of dopamine[11], has recently been demonstrated to occur in the gastropod nervous system, and this, with other data[12,13], suggests that dopamine receptors of gastropod nervous tissues may be identical with the dopamine-binding moiety of dopamine-sensitive adenylate cyclase, and that the effects of dopamine on the central nervous system may be due to dopamine-induced increases in adenosine 3′, 5′-monophosphate (cyclic AMP) in the post-synaptic cells. Strong evidence exists to support the opinion that cyclic AMP mediates the effect of dopamine in the mammalian superior cervical ganglion, retina and caudate nucleus[14] and also in the thoracic ganglia of the cockroach[15]. In view of the impressive data supporting a transmitter role for dopamine in the gastropod central nervous system, I examined more closely the effects of dopamine on cyclic AMP content with the aim of characterizing the receptors.

Material and methods. Active snails caught daily in the surrounding woods of Göttingen were used. Adenylate cyclase activity was measured in homogenates of tissue according to the method of Kebabian et al.[16] with some modifications. The standard assay system (final volume 500 µl) contained (in mmoles/litre): tris (hydroxymethyl) aminomethane-maleate 90; ATP, 0.5; Mg SO_4, 2.0; ascorbate, 0.5; theophylline, 5; EGTA, 0.2; plus test substances as indicated. The reaction was initiated by the addition of ATP. Incubation was for periods of 15 min in a



Increase in cyclic AMP in homogenates of snail central ganglia as a function of the concentration of dopamine and apomorphine. The data represent the mean ± SEM for 30 replicate samples in the case of dopamine, and 20 replicate samples in the instance of apomorphine. In the absence of added agonist, the cyclic AMP content was 2.78 ± 0.33 pmoles/mg wet weight of tissue (± SEM for 30 experiments).

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shaking water-bath at 25 °C. The reaction was terminated by placing assay tubes in a boiling water-bath for 3 min. The amount of cyclic AMP present in each tube was measured on 50 µl aliquots by the method of Gilman[17].

Results. In this study 350 µM dopamine caused maximal stimulation of adenylate cyclase in homogenates of snail central ganglia. In several experiments, the maximal increase was about 3fold. This agrees well with the value reported for dopamine-induced cyclic AMP formation in Aplysia ganglia[11]. The figure illustrates the effects of various concentrations of dopamine and also apomorphine, a dopamine agonist[18] on snail adenylate cyclase. The concentrations causing a half-maximal stimulation (EC_{50}) were approximately 50 µM for dopamine and 180 µM for apomorphine. In the vertebrates, half-maximal stimulations of adenylate cyclase for dopamine and apomorphine in slices of caudatus nucleus are in the region of 60 µM and 150 µM respectively[19]. The observation that apomorphine activates snail adenylate cyclase, but to a lesser extent than dopamine, is of particular interest, since Struyker Bondier et al.[20] have recently shown that apomorphine can mimic the excitatory effects of dopamine in snail neurons despite previous proof that it does not imitate the dopamine inhibitory effect in snail neurons[21].

At least 2 types of dopamine receptor have been reported to occur in the gastropod molluscs[6, 10]. Ergometrine selectively antagonizes the inhibitory effects of dopamine at moderate doses of about 10 µM[7], though at higher concentrations it has differential effects on the excitatory dopamine response[10]; and recent studies have also shown ergometrine to antagonize the inhibitory serotonin effects in gastropod neurons[5]. Excitatory effects of dopamine in the gastropod nervous system seem in contrast to be selectively blocked by tubocurarine and strychnine[22, 23]. Chlorpromazine and haloperidol, selective blockers of dopamine receptors in the vertebrates[24], have no effect upon the inhibitory dopamine responses but depress the excitatory responses in the gastropods[6, 7]. As shown in table 1, tubocurarine (100 µM) was found to antagonize the activating response of dopamine (120 µM) to snail adenylate cyclase almost completely, whilst ergometrine, chlorpromazine and strychnine at the same concentration (100 µM) only partly depressed the increase in the adenylate cyclase activity caused by 120 µM dopamine. Propranolol (100 µM) was without effect. Ergometrine at a concentration of 10 µM, which is known to depress selectively the dopamine inhibitory responses[7], had no effect on snail adenylate cyclase. However, haloperidol and fluphenazine, an antipsychotic agent of the phenothiazine class, blocked the increase in cyclic AMP response caused by dopamine at 100 µM. Haloperidol at 5 µM was not without effect, while fluophenazine still had a potent influence, even at 5 µM.

The ability of other substances to activate snail adenylate cyclase was also analyzed (table 2). Dose response curves for N-methyl dopamine and 5-hydroxytryptamine were obtained with EC_{50} values of 85 µM and 40 µM respectively with maximal stimulation of the same order as that for dopamine. The 5-hydroxytryptamine (120 µM) adenylate cyclase activating response was not inhibited by tubocurarine (100 µM), haloperidol (10 µM) or fluphenazine (10 µM). Isoprenaline, p-tyramine, octopamine, isoterenol, noradrenaline and histamine were all inactive on snail adenylate cyclase at 120 µM.

Discussion. The results reported here indicate that there are distinct dopamine receptors in the snail brain, the stimulation of which results in increased amounts of cyclic AMP. This supports the already impressive evidence that dopamine is a transmitter in the gastropod central nervous system. From the present data it would appear that adenylate cyclase is involved in the dopamine excitatory receptors, since drugs known to influence the dopamine excitatory responses, viz. tubocurarine, strychnine and haloperidol[6, 7, 22, 23], antagonize the dopamine-induced increase of cyclic AMP. In contrast, ergometrine,

Table 1. Effect of various substances on the dopamine stimulated adenylate cyclase in snail central ganglia

Substance	Cyclic AMP content (pmoles/mg wet weight tissue)
Dopamine (120 µm) alone	10.6±0.31
Dopamine (120 µm)+tubocurarine (100 µm)	2.9±0.29
Dopamine (120 µm)+tubocurarine (10 µm)	7.3±0.29
Dopamine (120 µm)+strychnine (100 µm)	7.8±0.33
Dopamine (120 µm)+chlorpromazine (100 µm)	8.8±0.29
Dopamine (120 µm)+propranolol (100 µm)	10.4±0.42*
Dopamine (120 µm)+ergometrine (100 µm)	8.3±0.28
Dopamine (120 µm)+ergometrine (10 µm)	10.8±0.33*
Dopamine (120 µm)+haloperidol (100 µm)	2.8±0.34
Dopamine (120 µm)+haloperidol (5 µm)	7.7±0.36
Dopamine (120 µm)+fluphenazine (100 µm)	2.6±0.34
Dopamine (120 µm)+fluphenazine (5 µm)	3.8±0.26

* Not significantly different from dopamine alone.
The basal level of cyclic AMP (without dopamine added to the incubation medium) was 2.9 ± 0.31 pmoles/mg wet weight tissue. Each value is the mean ± SEM for at least 10 experiments.

Table 2. Effect of dopamine and other substances on the stimulation of cyclic AMP production in homogenates of snail central ganglia

Substance	EC_{50} values for snail ganglia	EC_{50} values for striatum and caudate nucleus
Dopamine	50 µm	2 µm*, 160 µm**
Apomorphine	180 µm	150 µm**
5-Hydroxytryptamine	40 µm	–
N-Methyl dopamine	85 µm	1.5 µm*

Basal level of cyclic AMP in snail central ganglia was 2.7±0.29 pmoles/mg wet weight tissue (±SEM for 6 experiments).
* Values for striatum homogenates of rat, from Miller et al.[25]. ** Values for caudate nucleus slices of rat, from Forn et al.[19]. EC_{50} values refer to the concentrations required to give half maximal stimulation. ± Isoprenaline, p-tyramine, octopamine, isoterenol, noradrenaline and histamine were all without effect at 120 µm.

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known to block dopamine inhibitory effects[7,10], caused no alteration in the snail cyclic AMP. The snail dopamine receptors also seem to be similar to those of the vertebrates[19], since fluphenazine strongly counteracts the increase in cyclic AMP caused by dopamine, and apomorphine also mimics the effect of dopamine. Moreover, these receptors are unlike the β-adrenergic receptors of the vertebrates[19] in that they are not influenced by noradrenaline or propranolol. Although 5-hydroxytryptamine is more potent than dopamine in stimulating adenylate cyclase in the snail nervous system, which supports the results of Cedar and Schwartz[11], it appears as if the receptors for the 2 amines are different, since tubocurarine, fluphenazine and haloperidol had no influence upon the 5-hydroxytryptamine effect at concentrations which drastically inhibited dopamine stimulation of adenylate cyclase. To conclude, the present results provide support for the idea that a dopamine-sensitive adenylate cyclase may be the excitatory receptor for dopamine in the snail nervous system. Further studies on the biochemistry and pharmacology of this dopamine-sensitive adenylate cyclase and its relationship with physiological processes in neurons are, however, necessary not only to clarify the type of receptor, but also to locate their sites.

Investigation of the microstructure of kidney stones (oxalate type) by high voltage electron microscopy and electron diffraction

K. El-Sayed[1,2] and V. E. Cosslett

Department of Physics, Electron Microscopy Section, Old Cavendish Laboratory, Free School Lane, GB–Cambridge CB2 3RQ (England), 26 November 1976

Summary. The organic matrix of this type of urinary calculus contains 3 components, which differ in form and in amorphous/crystalline content. Deposition of crystalline material in the early stages of mineralisation seems to be epitaxially related to the orientation of the organic matrix.

Optical microscopy and X-radiography show that urinary calculi are composed of a series of layers of crystalline components and a lesser amount of organic matrix[3–5]. The crystalline layers contain oxalates and phosphates of calcium, varying in calculi of differing provenance. Boyce and Garvey[6] found that the matrix is made up of fibrils and amorphous interfibrillar material.

X-ray diffraction and microprobe analysis have been successful in investigating the nature and orientation of the crystalline regions[4,5,7] but electron microscopy has been little used, because of the difficulty of cutting sufficiently thin sections. Watson[8] applied a replica technique and obtained micrographs of hexagonal crystals from some specimens, provisionally identified as cystine. Catalina and Cifuentes Delatte[9] examined the calcium oxalate sediments deposited from urine, and obtained some electron micrographs and diffraction patterns. These showed a type of spherulitic crystal growth, but the diffraction patterns were diffused and probably dominated by organic matrix material deposited with the crystalline components[5].

Mohammed et al.[10,11] succeeded in obtaining thin sections of calculi and studied them by electron microscopy at 80 kV and by electron diffraction. They showed that the organic matrix is often arranged in layers alternating with crystalline components, but sometimes seems to be present in screw dislocated crystals. They were unable to distinguish structure in the organic matrix, or to find evidence for the nature of its role in crystal growth.

The availability of electron microscopes operating at very high voltage now makes it possible to study relatively thick sections, and advances in the techniques of microtomy facilitate the cutting of suitable sections from friable materials such as calculi. The present study utilized these developments to obtain detailed information on the structure of the organic matrix and its morphological relations with the crystalline regions.

Materials and methods. Sections were prepared from 3 stones collected by surgical operation from 3 different patients, 1 woman and 2 men. Some 200 sections, cut from all parts of a stone from the centre to the periphery, were examined in an AEI EM6B electron microscope at 80 kV and in the Cavendish high voltage microscope at 600 kV.

Embedding: Araldite was used as embeddiug material, several ratios of resin, hardener and accelerator being tried in order to obtain good coherent sections. Best results were obtained with a composition of 5 c^3 resin, 8 c^3 hardener and 0.4 c^3 accelerator, instead of that usually used for biological tissues: 10, 10 and 0.5 c^3 respectively.

Sectioning: Great difficulty was encountered in cutting and collecting thin sections from calculi. Glass knives prepared with different wedge angles were investigated. A 40° angle was found satisfactory for cutting, and allowed sections to be collected safely. Trouble was experienced from the water in the collecting trough wetting the surface of the stone so that the latter dragged sections with it during the motion of the microtome. To avoid this the level of the water in the trough must be lowered, and

1 Address: Dept. of Physics, Ein-Shams University, Cairo (Egypt).

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