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Dopamine: Its Occurrence in Molluscan Ganglia

Abstract. *Fluorometric and paper chromatographic evidence indicates that dopamine is the only catecholamine present in the ganglia of a number of lamellibranch and gastropod species.*

Dopamine has been identified in many vertebrate tissues, such as adrenal medulla (*1*), sympathetic nerve (*2*), brain (*3*), and lung (*4*), but it has never been clearly demonstrated in invertebrate animals. In assaying the ganglia of several molluscan species for catecholamines, I found substantial amounts of dopamine with little or no trace of norepinephrine or epinephrine.

The three sets of ganglia of each lamellibranch were dissected free and pooled, and, in gastropods, the entire circumesophageal ganglionic complex was removed. For each species used, the ganglia from several individuals were combined, weighed fresh, and assayed by the fluorometric method of Bertler *et al.* for epinephrine and norepinephrine (*5*); for dopamine, a modification (*3*) of the Carlsson technique (*6*) was used.

Table 1. Estimated concentrations of dopamine in molluscan ganglia. All weights are of fresh tissue. The first three species are gastropods. The others are pelecypods.

Assays (No.)	Av. wet wt. (mg)	Dopamine (μg/g)	
		Range	Av.
	Melongena corona		
3	51	51–82	63
	Lunatia heros		
3	119	12–38	27
	Busycon canaliculatum		
4	310	6–22	14
	Mercenaria mercenaria		
8	31	137–405	261
	Modiolus modiolus		
3	25	35–118	85
	Ensis directus		
3	27	31–49	37
	Mya arenaria		
1	11	—	96
	Mytilus edulis		
1	41	—	35
	Aequipecten irradians		
3	39	60–88	74
	Spisula solidissima		
2	42	26	26

The excitation and fluorescence spectra for the ganglia of every species sampled indicated that a substance fluorometrically identical with dopamine was present, but in no case were epinephrine or norepinephrine peaks clearly evident. In addition, paper chromatography of *Mercenaria* and *Busycon* ganglia, in which the technique of Bertler and Rosengren (*3*) and such solvents as phenol and 0.1*N* HCl or butanol, acetic acid, and water (4:1:5) were used, revealed a spot corresponding to the spot produced by standard dopamine. Again, epinephrine and norepinephrine were not detected. When the three *Mercenaria* ganglia (cerebropleural, pedal, and visceral) were assayed separately, high levels of dopamine were found in each ganglion, while fluorometric assay of other *Mercenaria* tissues such as gill, mantle, heart, and intestine failed to show any appreciable dopamine content. Thus it appears that dopamine, at least in *Mercenaria,* is concentrated in the ganglia and occurs in each of the three ganglia.

The concentration of dopamine can be estimated from microammeter readings taken at the excitation and fluorescence peaks, but these values (Table 1) only indicate relative orders of magnitude. Although the recovery of standard dopamine averaged 90 percent, I found that it could vary widely between the extremes of 68 and 136 percent. Nevertheless, these data demonstrate the relatively high concentrations of dopamine which appeared in the ganglia of every bivalve and gastropod mollusk sampled.

These results are consistent with the reported absence of epinephrine and norepinephrine from *Mytilus* (*7*) and with chromatographic evidence for dopamine in *Helix aspersa* mentioned by Kerkut and Walker (*8*). Östlund's survey of catecholamines in lower animals (*9*) is inconclusive for the mollusks, possibly because he used whole animal extracts.

In the past, dopamine has been regarded as merely the precursor of norepinephrine (*10*), but recent findings have suggested an additional, physiological role for this substance; possibly it is a neurohumor in mammalian brain (*3*) and in *Helix* brain (*8*), and it may function in the regulation of the *Mercenaria* (*Venus*) heart (*11*). This demonstration that dopamine is the principal catecholamine in molluscan ganglia also suggests that dopamine has a function independent of its role as the precursor to norepinephrine.

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11 January 1963

Ribonucleic Acid Synthesis in Protoplasts of Escherichia coli: Inhibition by Actinomycin D

Actinomycin D inhibits specifically and effectively DNA-dependant RNA synthesis in mammalian cells (*1*) and in several bacteria (*2*). *Escherichia coli* however, even in the form of spheroplasts, is resistant to the antibiotic (*3*). Since DNA-dependant RNA synthesis by *E. coli* extracts is sensitive to actinomycin (*3*), the resistance of intact *E. coli* bacteria might be attributed to impermeability of this organism to the drug. Because of the relevance of the actinomycin effect to the study of gene action, and in view of the unusual amount of information available about nucleic acid metabolism, phage infection, and the regulation of protein synthesis in *E. coli*, an actinomycin-sensitive *E. coli* system would be useful for studying these problems.

We have prepared protoplasts from

E. coli K 13 (*4*) and have found actinomycin D to be highly effective in suppressing the synthesis of RNA by these preparations. Cells were grown at 37°C either in minimal medium (medium A of Torrain, *5*) with 0.3 percent glycerol, or in Tryptone medium (*6*) with 0.1 percent glucose. Logarithmically growing cells (50 ml) were centrifuged and suspended in 1 ml of tris-sucrose (0.25*M* tris, *p*H 8.1, and 0.3*M* sucrose). Freshly dissolved lysozyme (60 μg) and ethylenediaminetetraacetic acid, *p*H 8 (6 μmole) were added (procedure modified from that of Mahler and Fraser, *7*). After 10 minutes at room temperature the protoplasts were resuspended in their original medium supplemented with 20 percent sucrose and 0.1 percent bovine serum albumin. The suspension of protoplasts was incubated at 37°C, and RNA synthesis was measured by determining the incorporation of C^{14}-uracil into an RNA fraction prepared according to the method of Schmidt and Thannhauser (*8*). To determine the effect of actinomycin on RNA synthesis, C^{14}-uracil was added 3 minutes after the addition of various doses of the antibiotic, and the protoplasts were incubated for 20 minutes. The relative amount of RNA synthesized during this period was measured; Fig. 1 shows that 0.2 μg of actinomycin per milliliter inhibited RNA synthesis almost completely.

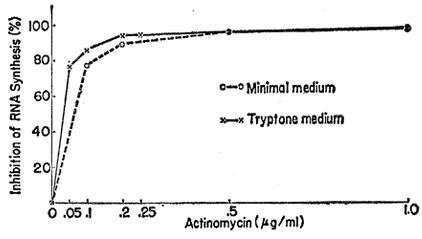

Fig. 1. Aliquots of protoplast suspension incubated for 20 minutes with various concentrations of actinomycin and with C^{14}-uracil (0.8 μc/ml, 0.3 μg/ml) were precipitated in the cold with $HClO_4$ (0.25*N*). The precipitate was washed three times with 0.25*N* $HClO_4$, then with ethanol-ether (1:3) and ether, and it was hydrolized in 0.5*N* KOH at 37°C for 18 hours. After neutralisation with $HClO_4$ the optical density of the supernatant was determined at 260 mμ in a Zeiss spectrophotometer, and the radioactivity of an aliquot measured in a Packard spectrometer. The ratio of the number of counts per minute to the optical density at 260 mμ for the untreated sample was considered 100 percent RNA synthesis. Each incorporation experiment was performed in duplicate.

The inhibition of DNA-dependant RNA synthesis in *E. coli* by actinomycin should prove to be a useful tool in the elucidation of such critical problems as the fate of phage informational RNA.

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5 March 1963

Hydrogen-Aluminum Clays: A Third Buffer Range Appearing in Potentiometric Titration

Abstract. *Wyoming montmorillonite (bentonite, particles 2 to 0.2 μ in diameter) treated with hydrogen-ion-saturated resin shows, on titration in 1N KNO_3 with NaOH by a continuously recording instrument, a third buffer range between* p*H 5.5 and 7.6 in addition to the first range where exchangeable hydronium is neutralized and the second range where a reaction with exchangeable aluminohexahydronium, $Al(OH_2)_6^{3+}$, occurs. The third range increases considerably when the hydrogen-ion-saturated clay is aged, and is attributed to basic aluminum compounds formed in the presence of negative charges of montmorillonite, comparable to "third range" buffering noted in aged, partially neutralized aluminum chloride solutions.*

Hydrogen-ion- (H) saturated montmorillonite changes to an aluminum-saturated form on aging (*1*). Among factors which influence the velocity of this transformation are temperature (*2*) and chemical composition of the clay (*3*). By this transformation, the electrostatically bonded hydronium associated with montmorillonite as a strong acid which functions in the first buffer range changes to aluminohexahydronium (*4*), which functions as a weak acid and exhibits the second buffer range, $pK_1 = 5$ (*5*). A still weaker acid group (third buffer range) on clays from soil has been indicated (*6*) by the considerable lime requirement that acid subsoils low in organic matter exhibit above the *p*H range in which monomeric aluminohexahydronium participates in exchange with KCl. This third buffer range had not so far been observed on potentiometric titration of hydrogen-aluminum clay minerals.

Recent potentiometric titration experiments with Wyoming montmorillonite (bentonite, particles 2 to 0.2 μ in diameter) treated with H-resin indicate that a very weak acid group appears as a third buffer range if the titration curve is made with a continuously recording instrument and in a fairly high concentration of electrolyte. The curves shown in Fig. 1 were obtained with a 0.5 percent suspension in 1*N* KNO_3 of montmorillonite treated with H-Amberlite IR-120, by means of a potentiograph provided with an automatic burette (Metrohm, Switzerland) and a titration rate of 0.0065 meq of NaOH per min, to 164 mg of clay. The third buffer range occurs, under these conditions, from *p*H 5.5 to 7.6 and is separated from those ranges where OH_3^+ and $Al(OH_2)_6^{3+}$, aluminohexahydronium, are neutralized. The third buffer range is detectable also after the clay is treated with potassium acetate of *p*H 5.4 instead of with H-resin.

The change of the titration curves, in particular that of the third range, has been followed during the aging of the montmorillonite suspension after treatment with H-resin. Figure 1 shows the curves after aging periods of 0, 18, and 71 hours, and 80 and 135 days, respectively.

The base consumption attributable to exchangeable hydronium and to aluminum as well as to the third range was estimated from the points of inflection of the curves and expressed as percent of total base consumption up to about *p*H 7.6 (Table 1). The percentage of exchange saturation with OH_3^+ decreased with time to almost zero; Al^{3+} first increased, then decreased. On the other hand, the third range increased throughout the aging period. The reversibility of this change in acid-group distribution was shown when the sample, aged for 135 days, was re-treated with H-resin. As the upper curve in Fig. 1 shows, the original curve was fully restored.