New Contributions to the Tone-Inhibiting Effect of Catecholamines in the Fresh-Water Mussel

In a previous paper[1], based on myographic and electrophysiological findings, we have already suggested that adrenaline and noradrenaline play a certain role in the inhibitory mechanism of the phenomenon described by PAVLOV[2].

In the present work we have determined the adrenaline and noradrenaline content in the tonic and tetanic portion of the posterior adductor, as well as in the nervous elements taking part in the regulation of the muscles in question, under normal conditions and also after transection of the cerebrovisceral connectives. From these results we wished to obtain new data on the physiological role of adrenaline and noradrenaline in the muscle processes of Lamellibranchiatae.

The determination of adrenaline and noradrenaline was carried out by two methods: the colorimetric method of MANUCHIN[3], and the fluorimetric method of EULER-FLODING[4]. Fresh-water mussels (*Anodonta cygnea*) served as laboratory animals. In each series, 10 animals were used.

The results of our experiments were as follows: After the transection of cerebrovisceral connective, connecting the cerebral ganglia with the visceral ganglia and the posterior adductor, the latter gradually developed a tonic contraction. When the catecholamine level of the visceral ganglia and of the posterior adductor was measured in the course of this process, an increase of the noradrenaline level in these tissues together with a simultaneous decrease of the adrenaline level was observed. (The changes indicated in the Table show the state of affairs 45 min after transection when full tone has developed.)

Analysis of the results mentioned above will show the following interesting relations: In the tonic portion of the posterior adductor both the adrenaline and noradrenaline level are somewhat higher than in the tetanic portion, at the same time the changes following transection of the cerebrovisceral connective are more pronounced in the former. This fact in itself indicates that catecholamines play a more important role in the regulation of tonic processes than in tetanic ones.

It is also remarkable that the noradrenaline level is only a mere fraction of the total catecholamine content. The latter statement may also be regarded as valid for the catecholamine content in nervous elements.

In the cerebral ganglia both the total catecholamine content and, within this, the adrenaline and noradrenaline level are higher than in the visceral ganglia. Considering that the cerebral ganglia are regarded as the inhibitory centre for tone of the posterior adductor muscle, this higher catecholamine level would seem to be indirectly indicative of the role of catecholamines in the tone-inhibiting processes.

Since after the transection of cerebrovisceral connective the inhibition of tone gradually weakens, evidently there must be a decrease in the intensity of one or more of the factors which are responsible for the processes of tone inhibition in physiological conditions and which are brought into play in the muscle processes by the cerebral ganglia.

Data in the literature[5,6] and our own observations show that catecholamines are capable of relaxing muscle tone. The decrease of adrenaline level seen in the present experimental results seems to indicate that adrenaline release, in physiological conditions, is one of the functions of the cerebral ganglia in the process of tone inhibition.

The posttransectional accumulation of catecholamine in the cerebral ganglia, though insignificant, seems to corroborate the above assumption.

The role of noradrenaline in this process can be disregarded because, on the one hand, in spite of its accumulation the tone develops normally, and on the other hand, exogenously applied noradrenaline produces an effect ten times as weak as that of adrenaline[6,7].

It could be assumed that the increase of the noradrenaline content with the parallel decrease of the adrenaline level indicates that in physiological conditions the function of the cerebral ganglia is to promote the methylation of noradrenaline to adrenaline, i.e. to an effective tone-inhibiting factor.

Concerning the disputable question of whether the fibres of the cerebral ganglia innervate the posterior adductor directly or only indirectly through the visceral ganglia, our present findings would seem to indicate that

Normal catecholamine level of ganglia in γ/g of wet tissues

Noradrenaline content of cerebral ganglia	Adrenaline content of cerebral ganglia	Noradrenaline content of visceral ganglia	Adrenaline content of visceral ganglia
0.56	5.36	0.38	4.0

Catecholamine level of ganglia after cutting of cerebrovisceral connective (the second line = changes in %)

0.62	5.5	0.75	1.9
$+10.7/P > 0.5/$	$+3/P > 0.5/$	$+97 P < 0.02/$	$-52.5/P < 0.002$

Normal catecholamine level of posterior adductor muscle in γ/g wet tissues

Noradrenaline content of tonic portion	Adrenaline content of tonic portion	Noradrenaline content of tetanic portion	Adrenaline content of tetanic portion
0.08	0.43	0.07	0.39

Catecholamine level of posterior adductor after cutting of cerebrovisceral connective (the second line = changes in %)

0.1	0.21	0.08	0.34
$+24/P > 0.5/$	$-51/P < 0.002/$	$+14/P > 0.5/$	$-13/P > 0.5/$

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these fibres – at least some of them – influence the visceral ganglia, because after the cutting of the cerebro-visceral connective the latter ganglia showed a significant change in catecholamine level.

Consequently, there seems to be numerous types of evidence to demonstrate the role of adrenaline played in the inhibition of tone; however, considering its inability to abolish tone completely, its role is regarded as that of an additive, facilitating factor. As formerly[7], serotonin is still thought to be a mediator in the inhibition of tone of the posterior adductor.

Zusammenfassung. Nach Durchschneiden des Cerebro-visceralkonnektivs nimmt der Adrenalingehalt im Visceralganglion und hinteren Schliessmuskel signifikant ab bei gleichzeitig leichter Noradrenalinzunahme. Die Änderungen sind in der tonischen Portion des Muskels ausgeprägter, was auf die Rolle des Adrenalins bei der Tonushemmung hindeuten dürfte.

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On the Action of Chromomycin on the Eggs and Embryos of *Ciona intestinalis*

Following the investigations of Gross and Cousineau[1,2] on the action of actinomycin D on sea urchin eggs, a number of workers have now used this antibiotic in order to study the MRNA metabolism in various eggs.

Recently, Wakisaka et al.[3] reported on a new antibiotic, chromomycin A_3, which is a powerful inhibitor of RNA synthesis in mammalian cells although it allows DNA synthesis to proceed. Thus the nature of its action is similar to that of Actinomycin D, and we therefore treated the eggs and embryos of *Ciona intestinalis* with solutions of chromomycin.

The Table summarizes the essential findings on unfertilized eggs, fertilized eggs and embryos.

These results show that the embryos become very sensitive to the action of the drug at the *late gastrula stage*, when a treatment of 1 h only is very effective. The effect, i.e. the abnormality in the larva, is very striking in the tail, which is much shorter than that of the normal larva. In addition, movement of the tail is very feeble and the larva is incapable of swimming. It therefore remains lying on the bottom of the culture dish. The sensory organs (visible as a pair of black dots in the normal larva) are also strongly hit, being fused and/or reduced in size. Further, the chordal cells are abnormally large, i.e. the process of cell division and differentiation has been blocked at an early stage.

	Concentration/ml	Period of treatment	Result
Unfertilized eggs	5–10 γ	3–5 h	No effect, i.e. normal larvae after fertilization
Fertilized eggs	10 γ	3 h	No effect
Embryos up to middle gastrula stage	3–5 γ	3 h	No effect
Late gastrula stage	3–5 γ	3 h	Marked effect, i.e. abnormal larvae
Late gastrula stage	10 γ	1 h	Marked effect
Late gastrula stage	10 γ	15 min	No effect

The effects of a certain substance on the development of an embryo is always difficult to interpret because the embryo is a unity in a dynamic state of continuous change, morphological as well as biochemical. Further, it is not known whether the substance is really specific or not. Chromomycin may have various non-specific actions but the supposition that it blocks RNA (including informational RNA) synthesis fits well with our results. We know that at the late gastrula stage protein synthesis is markedly evident and it is therefore quite likely that at this stage, RNA synthesis or the 'delivery of information' is much enhanced. If we accept this view, our results will mean that the system for releasing 'information' is not active in the unfertilized or early fertilized egg[4].

The details of this investigation, including a discussion on the permeability of eggs and sequential release of MRNA[5], will be published in Acta embryologiae et morphologiae experimentalis[6].

Riassunto. È stata studiata l'azione della cromomicina sulle uova di *Ciona intestinalis* (Ascidie) in diversi stadi di sviluppo. Essa non esercita alcun effetto sull'uovo vergine o appena fecondato; modifica invece notevolmente la morfogenesi se il trattamento è portato sulle uova in gastrulazione. I risultati vengono spiegati ammettendo che la cromomicina esercita la stessa azione che la actinomicina D e cioè inibisce la sintesi del MRNA.

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