Cholinomimetic drugs may affect growth and metamorphosis of the sea urchin larva

Cholinomimetic drug
Echinoderm
Paracentrotus lividus larvae
Growth
Metamorphosis

Substance cholinomimétique
Echinoderme
Larve de *Paracentrotus lividus*
Croissance
Métamorphose

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ABSTRACT

Cell-to-cell and cell-environment interactions are known to play a role during the first events of sea urchin development. Larval metamorphosis in benthic invertebrates is induced by environmental signals (cues), received by membrane receptors. The activity of such cues may be disturbed by the presence of toxic agents from pollution. We consider the possibility that neurotransmitter receptor-like molecules may have a role in the regulation of larval development and metamorphosis. To investigate the effects exerted on these processes by neuroactive pollutants, such as neurotoxic insecticides, we tested the function of acetylcholine receptors (muscarinic acetylcholine receptors mAChR and nicotinic acetylcholine receptors nAChR) in larvae of *Paracentrotus lividus* in the presence of four neuroactive drugs: *carbamylcholine*, an agonist of mAChR; *atropine*, an antagonist of the same; *nicotine*, an agonist of nAChR; and *eserine* (physostigmine), which mimics an excess of ACh in the receptorial sites.

Under the conditions of our experiments, the effect of these neuroactive drugs on larval development was mainly a growth delay in comparison with the development of controls. At metamorphosis, the neurotransmission system, which is predominantly cholinergic in larvae, shifts to a biogenic-amine system in the rudiment. At this stage, the weaker effects of the tested drugs show that their action was not exerted on transcription but rather on the regulation of motility in the ciliary bands.

RÉSUMÉ

Substances cholinomimétiques qui peuvent jouer un rôle de régulation au cours de la croissance larvaire et de la métamorphose de l'oursin.

Au cours des premiers événements du développement ontogénique de l'oursin, les neurotransmetteurs et leurs récepteurs sont responsables des interactions intercellulaires et de celles entre cellules et environnement. La métamorphose des larves d'invertébrés benthiques est souvent induite par des signaux de l'environnement captés par les récepteurs de membrane. Afin d'examiner les effets que peuvent produire sur la régulation de la croissance et de la métamorphose plu-

sieurs polluants neuroactifs comme les insecticides et les pesticides, nous avons testé sur les larves de *Paracentrotus lividus* l'activité des récepteurs de l'acétylcholine (récepteurs muscariniques mAChR et nicotiniques nAChR) en présence de quatre drogues cholinomimétiques: la carbamilcholine (agoniste du mAChR), l'atropine (antagoniste du méme récepteur), la nicotine (agoniste du nAChR) et l'ésérine qui simule un excès de ACh au niveau des récepteurs.
Dans les conditions de nos expériences les effets de ces drogues sur le développement larvaire se sont traduits principalement par un retard par rapport aux témoins. A la métamorphose le système de neurotransmission en majorité cholinergique laisse la place au système biogénique de l'imago. A ce stade les effets des concentrations les plus faibles indiquent que leur action ne se fait pas au niveau de la transcription mais plutôt sur la régulation de la motilité des bandes ciliées.

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INTRODUCTION

Larval metamorphosis constitutes the stage when organisms change their mode of life, environment, feeding, and behaviour. Pelagic larvae of echinoderms are carried by currents, so that embryos from the eggs of a single adult specimen migrate to wide areas in the sea, while after metamorphosis they change into benthonic specimens, able to move only short distances. For this reason, the choice of substrate is critical, and consequently metamorphosis is conditioned by the chemical properties of the substrate (1, 2, 3). Such behaviour derives from the ability to receive signals from the environment (cues), due to the exposure of membrane receptors. Furthermore, cell-to-cell and cell-environment signals are known to play a role in differentiation. We consider the possibility that neurotransmitter

Table 1

Effects caused on sea urchin development by drugs at different concentrations.

Drug	Concentr.	Time of exposure:			
		Since hatching			Initial metamorphisis
		4 arm stage	8 arm stage	metamorphosis	metamorphosis
atropine	10^{-5} M	lethal anomalies, 30% survival	delay of development in surviving embryos	none	none
	10^{-8} M	thin and long arms 78% survivial	thin and long arms, small gut, small rudiment	delayed	delayed, small rudiments
	10^{-12} M	thin and long arms 85% survival	thin and long arms, small gut, small rudiment	at the same time as controls	delayed
carbachol	10^{-5} M	anomalous larvae, 20% survival	heavy anomalies	none	delayed
	10^{-8} M	65% survival	short and thick arms, big rudiments, delayed dev.	strongly delayed	delayed, big skeleton
	10^{-12} M	85% survival	short and thick arms, big rudiments, delayed dev.	delayed, big juvenile, with well developed skeleton	delayed, big skeleton
nicotine	10^{-5} M	15% survival	death of 100% larvae	none	none
	10^{-8} M	anomalous gastrulation, 30% surviving thin plutei	slight animalization of larvae	delayed	delayed, small juvenile
	10^{-12} M	80% normal larvae, strong ChE activity	normal larvae, strong ChE activity	delayed	delayed
eserine	10^{-5} M	lethal anomalies, 20% survival	anomalies, 15% of normal larvae	short delay	delayed
	10^{-8} M	45% survival	thick larvae, short arms, big rudiments, delayed dev.	none: death of 100% larvae	delayed
	10^{-12} M	70% survival	thick larvae, big rudiments, delayed dev.	slightly delayed, big juvenile	delayed, big skeleton.
controls	—	97% survival	65% survival	30% survival	

receptor-like molecules may have a role in the regulation of larval growth and metamorphosis, as neurotrasmitters have been found responsible for cell interactions during early morphogenetic events of different organisms, including sea urchins (4, 5). In this case, great relevance should be accorded to the effects exerted on the environment by most insecticides, pesticides, and generally neuroactive pollutants originating at agricultural and manufacturing sites as well as toxicants dispersed as aerial sprays (6). To this end, we carried out preliminary tests on the function of acetylcholine (ACh) receptors in metamorphic induction. These receptors belong to two different families: muscarinic receptors (mAChR), which trigger a signal transduction system by activing an intracellular G-protein; and nicotinic receptors (nAChR), which, after excitation by the ligand, open a Na^+ channel.

MATERIALS AND METHODS

Four ACh receptor-active drugs were tested to determine whether these receptors are involved in the regulation of development and metamorphosis: (I) *carbamylcholine* (carbachol), an agonist of mAChR; (II) *atropine,* an antagonist of the same receptor; (III) *nicotine,* an agonist of nAChR; (IV) *eserine,* which mimics an excess of ACh, because it is an inhibitor of cholinesterases (ChE), which detach ACh, or choline esters, from AChR. Further, we tested *actinomycin D,* an antibiotic able to inhibit mRNA transcription during the G1 phase. It is known from the literature that *actinomycin D* inhibits early sea urchin development from the start of gastrulation. In this study, *actinomycin D* (180 µg/ml) was added to sea water containing larvae at the eight-armed stage, both before and after the

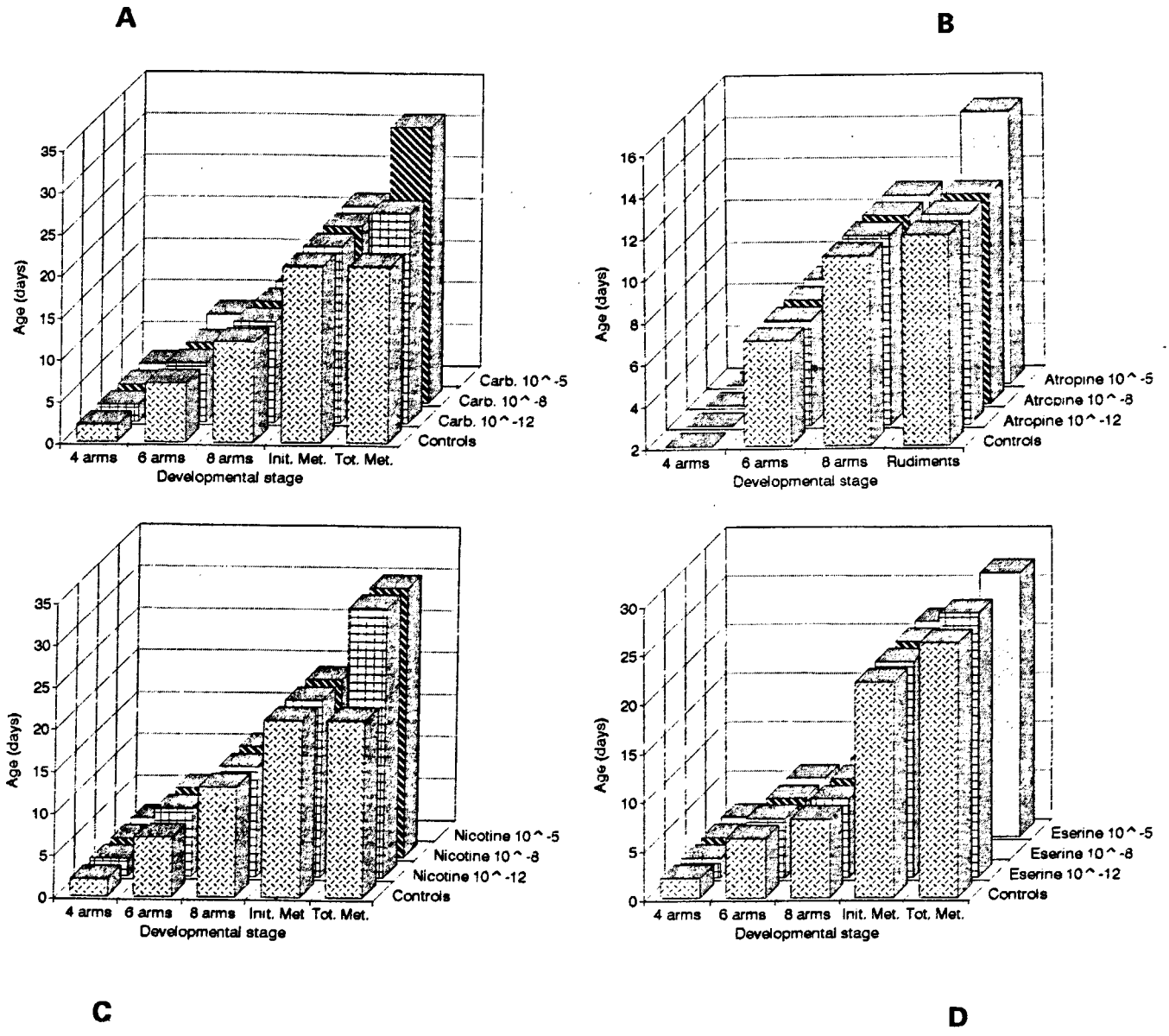

Figure 1

Duration of development (in days) of embryos exposed from hatching to different concentrations of A) carbachol; B) atropine; C) nicotine; D) eserine. Exposure since hatching.

Durée du développement (en jours) des larves traitées avec différentes concentrations de A) carbachol; B) atropine; C) nicotine; D) ésérine.

beginning of metamorphosis, in order to determine the level at which the cholinomimetic drugs can affect the regulation of gene expression.

Gametes were obtained in the laboratory from *Paracentrotus lividus* specimens; the experiments were repeated five times, in order to obtain larvae from different parents, and in different periods between November 1991 and May 1992. Drugs were dissolved in the rearing sea water which was changed at two-day intervals. Drugs were applied either from the beginning of development (immediately after hatching) until metamorphosis, or at the beginning of metamorphosis, before the appearance of rudiments. Drug concentrations ranged between 10^{-5} and 10^{-12} M; larvae were sampled and fixed at regular intervals.

RESULTS AND CONCLUSIONS (Table 1)

None of the tested drugs, applied at any concentration, was able by itself to induce metamorphosis. All the drugs caused damage and delay in metamorphosis, but not always in larval growth, which was positively influenced by mAChR-agonist drugs (carbachol and eserine). Drugs applied at higher concentrations (10^{-5} M) caused high mortality and often lethal anomalies. At 10^{-12} M, mortality was lower; no pronounced anomalies were observed, but in all cases development was delayed as compared to controls (Fig. 1).

Drug concentrations between 10^{-8} and 10^{-9} M: in the larval stages, *carbachol* accelerated development up to the prism stage, with a noticeable increment of the skeletal spiculae, and a slight vegetalization of the four-arm stage. *Nicotine*-treated gastrulae exhibited gut evagination and lysis, followed by high mortality (about 85 % of the gastrulae). The surviving embryos presented a slight animalization, with thin spiculae and elongated arms with long cilia. *Atropine*-treated larvae exhibited elongated arms and a small gut, with thick walls.

At the end of metamorphosis, the last larval structures to disappear are the ciliate epaulettes that contain both sensory (7) and swimming structures; the preminently cholinergic neurotransmitter system of the larva shifts to a biogenic-amine system of the imago (shown by the FIF reaction, Fig. 2). At this stage the cholinomimetic drugs have lesser effects (demonstrated by the experiments of drug application after the beginning of metamorphosis).

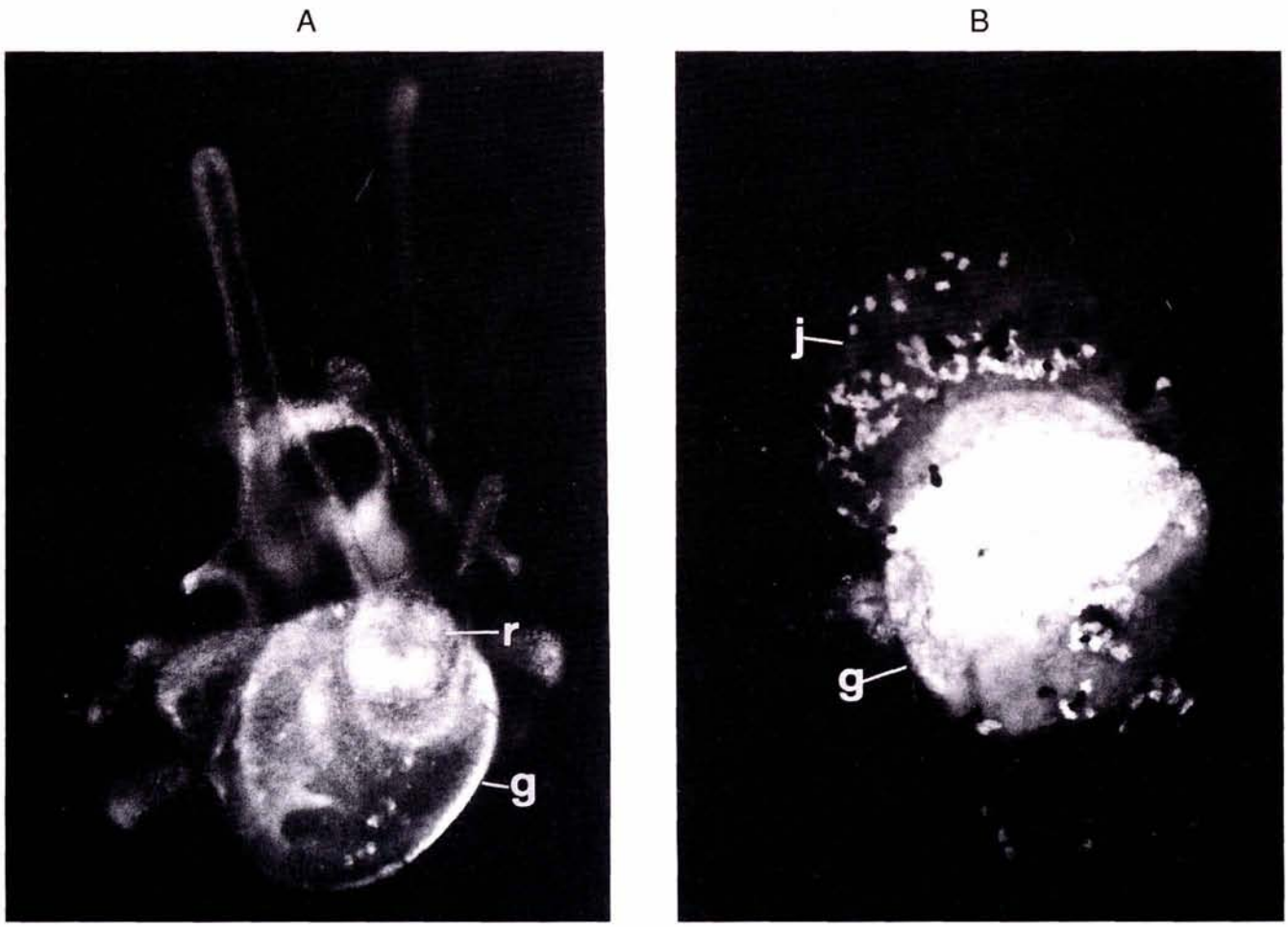

Figure 2

FIF (formaldehyde-induced fluorescence) obtained A) in eight-arm larvae treated with 10^{-12} M atropine and B) in the corresponding control larvae. g = gut; r = rudiment; j = juvenile.

FIF (formaldehyde-induced fluorescence) dans : A) larve traitée avec atropine 10^{-12} M ; B) témoin correspondant. g = intestin; r = rudiment; j = juvénile.

Drug concentration 10^{-12} M: in the experiments with drug application from the beginning of development, although no heavy anomalies were exhibited by either larvae or rudiment stages, shape and size differences were present, and varied according to the type of drug action. *Atropine* caused miniaturized ciliate epaulettes and rudiment, while the larval arms appeared elongated and thinner as compared to the control larvae (at 18 days the ratio between arm length and rudiment diameter was 8.2 ± 0.3, while in controls it was 4.20 ± 0.8). The gut appeared rather empty, and reduced in volume. If the drug was applied at the beginning of metamorphosis, the effect exerted on the larval size of was less evident.

Carbachol and *eserine* produced quite different effects: the ciliate epaulettes and the rudiment appeared bigger and more developed than in controls, (the ratio arm length/rudiment diameter was 3.15 ± 0.5). For the particular effects of both *carbachol* and *eserine,* this finding may agree with the fact that in the vertebrate CNS there is a correlation between the levels of ACh and nerve growth factor (NGF). The gut was big and full of algae. *Carbachol,* applied at any time, caused faster metamorphosis (Fig. 1) and early appearance of calcareous spines of the juvenile, perhaps indicating a faster synthesis of glycoproteins enhancing inorganic salt deposition (5). Larval structures, particularly the ciliary bands in both control and treated samples, always presented a positive ChE reaction, also in eserine-treated samples. This finding shows that a rapid turnover of the enzyme is present in living structures. When the eserine treatment was performed on fixed specimens, no ChE reaction took place.

At the higher concentrations (10^{-5}-10^{-8} M), nicotine caused severe damage to both larvae and rudiments; it caused a low lethality rate and a short delay in metamorphosis when used as diluted as 10^{-12} M.

Actinomycin D, applied after the beginning of metamorphosis, did not affect the process, indicating that transcription of mRNA is early, and probably due to the first interaction between ectoderm and hydrocoel cell sheets that takes place at the eight-armed stage. This early interaction may be considered as an inductive signal, so that the growth of the juvenile and the signal for larval breakdown seem to be independent of transcription and to depend on other causes probably linked to the environment. In this case, the effects of cholinomimetic drugs during late metamorphosis are not due to development regulation at the transcriptional level, but rather to the regulation of motility in ciliary bands that are the last to disappear. Thus, the different shape and size of larvae and juveniles may be due to the feeding activity of the arm cilia, which are affected in different ways by the drugs, as previously described (8, 9).

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