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Amyloid Precursor Protein 96–110 and β -Amyloid 1–42 Elicit Developmental Anomalies in Sea Urchin Embryos and Larvae that are Alleviated by Neurotransmitter Analogs for Acetylcholine, Serotonin and Cannabinoids

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Abstract

Amyloid precursor protein (APP) is overexpressed in the developing brain and portions of its extracellular domain, especially amino acid residues 96–110, play an important role in neurite

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outgrowth and neural cell differentiation. In the current study, we evaluated the developmental abnormalities caused by administration of exogenous APP_{96–110} in sea urchin embryos and larvae, which, like the developing mammalian brain, utilize acetylcholine and other neurotransmitters as morphogens; effects were compared to those of β -amyloid 1–42 (A β 42), the neurotoxic APP fragment contained within neurodegenerative plaques in Alzheimer's Disease. Although both peptides elicited dysmorphogenesis, A β 42 was far more potent; in addition, whereas A β 42 produced abnormalities at developmental stages ranging from early cleavage divisions to the late pluteus, APP_{96–110} effects were restricted to the intermediate, mid-blastula stage. For both agents, anomalies were prevented or reduced by addition of lipid-permeable analogs of acetylcholine, serotonin or cannabinoids; physostigmine, a carbamate-derived cholinesterase inhibitor, was also effective. In contrast, agents that act on NMDA receptors (memantine) or α -adrenergic receptors (nicergoline), and that are therapeutic in Alzheimer's Disease, were themselves embryotoxic, as was tacrine, a cholinesterase inhibitor from a different chemical class than physostigmine. Protection was also provided by agents acting downstream from receptor-mediated events: increasing cyclic AMP with caffeine or isobutylmethylxanthine, or administering the antioxidant, α -tocopherol, were all partially effective. Our findings reinforce a role for APP in development and point to specific interactions with neurotransmitter systems that act as morphogens in developing sea urchins as well as in the mammalian brain.

Keywords

Acetylcholine; Amyloid Precursor Protein; β -Amyloid; Cannabinoids; Development; Sea urchin embryo; Serotonin

INTRODUCTION

The accumulation of plaques containing β -amyloid protein is a major feature of Alzheimer's disease [38,40] and proteolytic cleavage produces fragments, most notably β -amyloid peptide 1–42 (A β 42), that possess distinct cytotoxic and neurotoxic properties [24,44,50,56]. Nevertheless, recent work points to important roles of amyloid precursor protein (APP) in neurodevelopment, neural cell migration, synaptogenesis and synaptic plasticity, connoting an important neurotrophic role [35,37,48,53]. Indeed, although APP is an integral membrane protein, a specific 15 amino acid sequence in positions 96–100 (NWCKRGRKQCKTHPH), located within the extracellular domain of the parent protein, represents a proteoglycan-binding domain that specifically controls neurite outgrowth and other aspects of neurodevelopment [45]. Accordingly, introduction of the free peptide itself interferes with the natural neurotrophic functions of APP, resulting in impaired neuritogenesis [45]. APP thus joins other neuropeptides that have unique functions in the developing brain, such as acetylcholinesterase, which similarly plays a structural role in neurite growth [23,47], and opioid growth factor, which negatively modulates neural cell replication and growth [54,55]. What is especially interesting about the trophic role of APP is its relationship to specific neurotransmitter systems, including acetylcholine (ACh) [25], which itself has neurotrophic properties in the developing brain [28,32].

In the current study, we used the developing sea urchin embryo to evaluate the morphogenetic effects of APP_{96–100} in contrast to already-identified actions of a different A β 42 [13,20], the neurotoxic APP fragment involved in plaque formation. The sea urchin embryo, develops the ability to synthesize, store and release ACh and other neurotransmitters, and possesses the analogous receptors and downstream signaling cascades, all of which appear rapidly over a defined developmental period and act as morphogens that control cell differentiation and assembly of the embryo and larva [10,13,20,26]. Agents that target specific neurotrophic mechanisms or signaling cascades in the mammalian brain, produce structural anomalies

during sea urchin morphogenesis that are readily characterized with routine light microscopy [4,5,9–12,17–19,36,41]. Further, normal sea urchin development is well characterized at both structural and biochemical levels; consequently, the mechanisms of action and consequences of exposure for a wide variety of embryotoxins, teratogens and neurotoxicants have been evaluated with this system [5,9,11,12,16,19,27,36]. Unlike mammalian models, each sea urchin produces thousands to millions of offspring that develop rapidly and simultaneously, with a coordinated and highly specific sequence of morphological events, so that toxicant exposures produce uniform phenotypes for any given critical exposure period [11,12,19,36].

We [13] and others [20] recently showed that A β 42, the β -amyloid fragment suspected to cause neurotoxicity in Alzheimer's Disease, elicits dose-dependent malformations in sea urchin embryos and larvae. Further, we found that membrane-permeable neurotransmitter analogs could prevent the adverse effects of A β 42 [13]; although analogs of ACh, dopamine, serotonin (5HT) and cannabinoids all offset the toxicity, those targeting ACh systems were the most effective. Here, we performed a comparative study for APP_{96–110}, adopting the same strategy used to identify its role in neurodevelopment in higher organisms [45], namely to add soluble, exogenous peptide so as to preempt the role of endogenous, full-sequence, membrane-anchored APP. As in our earlier work [13], we evaluated the protective effects of ACh, 5HT and cannabinoids by using analogs synthesized with arachidonoyl moieties to enhance lipophilicity as required to penetrate into the embryo, focusing on agents whose biological activities against more classical neurotoxicants were verified in earlier work [2,6,7,11–13]. These were compared to other neurotransmitter-related chemicals and in addition, we evaluated agents acting through other mechanisms that could impact neurotrophic effects or downstream neurotoxic consequences of APP_{96–110}: cholinesterase inhibitors, phosphodiesterase inhibitors, α -adrenergic and NMDA receptor antagonists, and antioxidants.

MATERIALS AND METHODS

Adult specimens of *Sphaerechinus granularis* were collected by scuba in the Adriatic Sea and were immediately transported (maximum 1 hr transportation time) to the Institute for Marine Biology (Kotor, Montenegro) for experiments. The optimal conditions for maintaining the adults and embryos have been described in detail previously [9,14,16,19]. In brief, gametes were harvested and the eggs were fertilized and incubated using standard techniques [12,16, 19]. Eggs or sperm were obtained by injecting the animals with 1 ml of 0.55 M KCl, and embryos and larvae were cultured at 21°C in artificial seawater (ASW; 445 mM NaCl, 24.6 mM MgCl₂, 18.1 mM MgSO₄, 9.2 mM KCl, 2.36 mM NaHCO₃, 11.5 mM CaCl₂; pH 7.4), maintaining complete O₂ saturation. Fertilization of the eggs was carried out using the combined sperm of 2–3 males for each female. We did not eliminate the fertilization envelope with p-aminobenzoic acid because we found in preliminary experiments that the envelope did not interfere with the effects of A β 42, APP_{96–110} or the neurotransmitter analogs. We used the fertilized eggs from 14 females, and in each experiment, eggs derived from any given female were equally distributed to all the treatment groups so as to enable matched comparisons. Because we needed <1% of the total number of fertilized eggs from any given female, the surplus embryos, larvae and adult sea urchins, were returned to the sea to help maintain the population at the site of origin.

The embryos or larvae were placed in 12-well cell culture plates at a concentration of approximately 100 embryos per ml of suspension per well. Test substances were introduced into the wells at three specific stages: the 2–4 cell stage (1 hr 20 min – 2 hr 30 min after the fertilization of the eggs); at the mid-blastula 2 to late blastula 1 stages (10–13 hr post-fertilization); or the late gastrula to early pluteus stages (32–36 hr post-fertilization). Embryonic and/or larval phenotypes were documented with digital photography/videography using a VANOX Olympus Universal Research microscope. Examinations began at 2–4 hr after

the start of treatment, with image capture occurring at varying intervals as required to document the effects. In each case we matched the appropriate control wells to the wells containing embryos exposed to the various test agents. A β 42 (oligomeric peptide; Chemicon International, Temecula, CA) and APP_{96–110} (Ac-NWCKRGRKQCKTHPH–NH₂, disulfide bond 3–10; AnaSpec, San Jose, CA) were used at concentrations from 0.1 to 1 μ M or 0.5 to 10 μ M, respectively, after preliminary studies established these ranges as appropriate to evaluations for threshold to maximal effects. These concentration ranges are similar to those shown to be required for A β 42 toxicity in other lower organisms [20,22,30,33,51]. Lipophilic analogs of ACh (arachidonoyl dimethylaminoethanol, AA-DMAE), 5HT (arachidonoyl 5HT, AA-5-HT), and cannabinoids-vanilloids (arachidonoyl vanillylamine, AA-VAN) were evaluated at 10–40 μ M, a concentration range found to be optimal in our previous experiments with a variety of neurotoxins [12,15,19], including A β 42 [13]. These substances were synthesized in the laboratory of Dr. V.V. Bezuglov [1,2] and provided at 98% purity in stock solutions of 10–20 mg/ml in ethanol. For the experiments, each stock solution was diluted in ASW to achieve a final concentration of 0.1–0.2% ethanol, which was also included in the control incubations and which did not adversely affect development by itself. Protective agents were introduced 5–10 min prior to adding A β 42 or APP_{96–110}.

The arachidonoyl neurotransmitter analogs were compared to a number of commercially-available agents targeting the same pathways: nicotine (Sigma Chemical Co., St. Louis, MO) and d-tubocurarine (Sigma) for ACh systems, and 5HT (Sigma) and N,N,N-trimethyl 5HT (SHTQ; ICN, Costa Mesa, CA) for 5HT systems. In addition, we compared the effects to other neurotransmitter-related agents: cholinesterase inhibitors, tacrine and physostigmine (both from Sigma); xanthine-based agents possessing adenosine receptor antagonist and phosphodiesterase inhibitor characteristics, caffeine (ICN) and 3-isobutyl-1-methylxanthine (IBMX, Sigma); an α -adrenergic blocker, nicergoline (Sigma); an antioxidant, α -tocopherol (from Sigma); and an NMDA receptor antagonist, memantine (Sigma). These agents were prepared in stock solution using ethanol, methanol or distilled water, and again diluted in ASW to final vehicle concentrations of 0.1–0.2%, with the same vehicle used for controls. Within an experiment, each of the adult females provided embryos for all treatment groups so that comparisons of treatment effects could be made within the same cohort of embryos.

RESULTS

Because the treatment effects were uniform across cohorts of embryos from all the individual adults, results are shown as representative photomicrographs (magnification 100–200 $\times$). Given that the thousands of embryos in each treatment group had virtually identical phenotypes for a given treatment, and that malformed embryos were never seen in the control groups, statistical analyses were restricted to simply assessing the proportions of wells showing malformations in the treated vs. control groups, regarding the embryos in a given well as a single sample. Using Fisher's Exact Test, all treatment effects were significant at $p < 0.00001$. As discussed below, the threshold defines the lowest concentration at which malformations are manifest, and with increasing concentrations, the malformations get progressively worse in appearance, culminating in total arrest of embryonic development and then embryonic death at the higher concentrations.

Detailed descriptions of sea urchin embryo development and the classifications of various malformations have been published previously [11,19], and many of the effects seen here for APP_{96–110} are identical in appearance to those reported earlier for adverse effects of A β 42; accordingly, only a few representative micrographs are shown, with the net results summarized in tabular form. For each set of micrographs, we show results from A β 42 and APP_{96–110} concentrations chosen to lie just above the threshold for rapid blockade of development,

whereas the protective agents were used at maximally-effective concentrations based on our previous work [13] or as established in preliminary dose-effect studies.

In our earlier work, we found malformations resulting from submicromolar concentrations of A β 42, added at any stage of embryogenesis from the first cleavage divisions up to the formation of larvae (plutei), culminating in arrested development and embryonic death [13]. The mid-blastula stage showed the lowest threshold, compatible with a peak of sensitivity. In the current study, we repeated this finding and compared the actions of APP₉₆₋₁₁₀ by conducting detailed dose-response evaluations at all the same stages. As shown in Figure 1, considerably higher concentrations of APP₉₆₋₁₁₀ were required to obtain malformations and there was a much more restricted window of vulnerability, with abnormalities elicited only with APP₉₆₋₁₁₀ added between the mid-blastula to mid-gastrula stages. Before or after this period, even concentrations as high as 8–10 μ M had no effect. Within the vulnerable period, though, the outcomes from APP₉₆₋₁₁₀ exposure were similar to those seen with A β 42 [13], with so-called “occluded” blastulae, gastrulae or prisms [13,20]. At the lowest effective concentration of APP₉₆₋₁₁₀ (0.5 μ M), we often found cells released into the blastocoele, accumulating initially near the apex simultaneously with thinning of archenterons, and only later filling this cavity (not shown).

We then used the critical period of sensitivity to examine potential neurotransmitter-related interventions to prevent or offset the adverse effects of APP₉₆₋₁₁₀. Because lipophilic analogs of ACh and 5HT were found to be highly effective against A β 42 [13], we first compared the actions of AA-DMAE and AA-5HT to 5HT itself and the hydrophilic analog, 5HTQ. Both of the lipophilic neurotransmitter derivatives prevented the adverse effects of APP₉₆₋₁₁₀ added at the mid-blastula-2 stage, whereas 5HT had only a marginal effect and 5HTQ had no protective action (AA-5HT and 5HTQ shown in Fig. 2). We next compared AA-5HT and nicotine against both A β 42 and APP₉₆₋₁₁₀, added at the mid-blastula-2 to late blastula-1 stage (Fig. 3). For A β 42, the toxicant arrested embryonic development and produced obvious malformations through the early gastrula-2 stage, after which the embryos died; either AA-5HT or nicotine produced strong to complete protection against A β 42 (Fig. 3, top panels). For APP₉₆₋₁₁₀, we found the same malformations at the early gastrula-2 stage (not shown) but the embryos maintained their viability, so our evaluations continued through the early pluteus stage; both agents again provided clear-cut protection (Fig. 3, bottom panels).

We then expanded the scope of the studies to include a wide variety of agents targeting different neurotransmitter systems and signaling pathways, adding each test substance along with APP₉₆₋₁₁₀ at the mid-blastula-2 to late blastula-1 stage and examining the outcome at the early pluteus stage (Fig. 4). Again, both AA-DMAE (not shown) and AA-5HT provided a high degree of protection at concentrations ranging from 10–40 μ M, with lesser protection from the lipophilic cannabinoid agonist, AA-VAN (not shown). Looking at other ACh-related drugs, nicotine was also effective but the cholinesterase inhibitor, physostigmine, was less so, and d-tubocurarine was only barely effective. Tacrine, another cholinesterase inhibitor, by itself caused sufficient damage to sea urchin development that the embryos were nonviable, and thus could not be evaluated for protection against APP₉₆₋₁₁₀; similarly, nicergoline and memantine proved directly toxic to developing sea urchin embryos (not shown). Both caffeine and IBMX provided partial protection, somewhat inferior to that from AA-DMAE but similar to AA-5HT, with a better response from IBMX. α -Tocopherol also partially protected the embryos and larvae from the adverse effects of APP₉₆₋₁₁₀.

DISCUSSION

The proteolytic cleavage fragment of β -amyloid protein, A β 42, has well-characterized neurotoxic properties [24,44,50,56] that are paralleled by its ability to disrupt sea urchin

embryogenesis [13,20]. As shown here, APP₉₆₋₁₁₀ also shares the ability to disrupt development but with two important differences. First, APP₉₆₋₁₁₀ was less potent, requiring concentrations an order of magnitude higher to produce the same malformations elicited by A β 42. Second, APP₉₆₋₁₁₀ acted only within a defined window of vulnerability centered around the mid-blastula stage, whereas A β 42 had adverse effects at all stages ranging from the earliest cleavage divisions through late pluteus [13,20]. Indeed, the peak period of sensitivity to APP₉₆₋₁₁₀ is also the period in which agents that interfere with the trophic actions of neurotransmitters exhibit their greatest potency in causing malformations [5,9,11,12,16,19, 27,36], suggesting a convergence of these disparate agents on a common set of end pathways regulating embryogenesis. APP₉₆₋₁₁₀ has neurotrophic activity that is important for its participation in brain development and plasticity [35,37,45,48,53] particularly so for ACh systems [25]. In keeping with this interpretation, membrane-permeable ACh agonists were the most effective agents offsetting the adverse effects. However, ACh analogs were not the only agents that reversed or prevented the effects of either of the amyloid sequences. Indeed, the most effective chemicals center around three distinct targets: ACh, 5HT and cyclic AMP-related mechanisms. AA-DMAE and nicotine, both membrane-permeable ACh agonists, provided effective protection, even to the point of restoring completely normal development. In contrast, d-tubocurarine, which is an antagonist at the neuromuscular subtype of nicotinic ACh receptors, but also has partial agonist properties, was less effective; this may reflect a lack of homologous correspondence between nicotinic receptors in the sea urchin and this particular mammalian subtype, or alternatively could reflect predominance of antagonist, rather than partial agonist properties of d-tubocurarine. Physostigmine, a cholinesterase inhibitor, also proved somewhat protective, likely through indirect enhancement of ACh function as a result of impaired ACh breakdown.

Nevertheless, cholinesterase inhibitors were not uniformly beneficial. Tacrine proved to be directly toxic to developing sea urchin embryos, and chlorpyrifos, an organophosphate pesticide that inhibits cholinesterase, is similarly known to produce developmental malformations [7,12,14,19]. These agents, although all sharing anticholinesterase activity, actually have very different structures and belong to separate classes of cholinesterase inhibitors. Cholinesterase is now known to play a nonenzymatic, structural role in neural assembly [3,31], and the disparities seen here in the sea urchin model could thus reflect different conformational changes in cholinesterase engendered by its interaction with structurally-diverse inhibitors. Alternatively, each of these agents also possesses properties other than cholinesterase inhibition that can contribute to adverse outcomes. For example, chlorpyrifos interacts directly with nicotinic ACh receptors to block its channel function [29,46], and also interferes with cell signaling pathways that provide important developmental cues, notably that involved in the generation of cyclic AMP [21,42,43,52]; these effects are opposite to those elicited by AA-DMAE and theophylline or IBMX, and would therefore have an adverse impact on development. In turn, then, the sea urchin model can contribute to understanding how different structural features might contribute or detract from the protective potential of compounds in different classes.

The fact that the very same protective agents worked in preventing the actions of A β 42 and APP₉₆₋₁₁₀, with virtually the same rank order of effect, argues strongly for convergence of these agents on common final pathways that affect morphogenesis (summarized in Table 1). Just as for the membrane-permeable ACh analogs, AA-5HT provided major protection against A β 42 or APP₉₆₋₁₁₀, echoing earlier findings with A β 42 or otherwise unrelated toxicants (Table 1) such as chlorpyrifos [7,12,14,19], or reserpine [11,14]. There are important implications of the fact that either ACh or 5HT analogs rescue the developing embryos from such apparently diverse agents. Although it is true that A β 42, APP₉₆₋₁₁₀, chlorpyrifos and reserpine all target ACh and 5HT pathways to differing extents, it is surprising that an ACh analog should rescue embryos from the effects of reserpine, which acts primarily by depleting monoamines such as

dopamine and 5HT. Further, AA-VAN, which acts through cannabinoid/vanilloid receptors, also provided partial protection against all four toxicants. It is highly unlikely that ACh, 5HT and cannabinoids all ameliorate the adverse effects of A β 42 and APP₉₆₋₁₁₀ through the same exact mechanism. For example, activation of CB1 cannabinoid receptors blocks β -amyloid toxicity through events far downstream from the receptors rather than interfering directly with its actions [24]. Cannabinoids also protect neurons from adverse effects of organophosphate pesticides [34], actions which we have reproduced in the sea urchin embryo (Table 1). One possibility is that disparate agents converge on common final signaling pathways to offset the effects of toxicants that act through different originating mechanisms but that ultimately compromise these downstream functions. In the current study, we tested that hypothesis through the use of two agents, caffeine and IBMX, that enhance cyclic AMP formation through their shared ability to inhibit phosphodiesterase. Both were effective, with IBMX working somewhat better than caffeine; since IBMX is more potent than caffeine as a phosphodiesterase inhibitor but less potent as an adenosine receptor blocker (a second property of xanthine derivatives), our results point to the cyclic AMP-related mechanism as the important property. This is reinforced by the ability of these agents to offset the adverse effects of chlorpyrifos, which also compromises cyclic AMP production as an important component of its disruption of development [21,42,43,52]. We also saw a beneficial effect of an antioxidant, α -tocopherol, which is likely to reflect actions on oxidative stress, well downstream from the primary effects of the amyloid peptides or the other toxicants shown in Table 1.

We also tested neurotransmitter receptor blocking agents as negative control agents, since they would seem highly unlikely to be effective against A β 42 or APP₉₆₋₁₁₀. Memantine acts in part through blockade of NMDA receptors, which are known to be important for brain assembly [49]. Not surprisingly, then, memantine by itself proved embryotoxic in the sea urchin model. Similarly, nicergoline acts as an α -adrenergic blocking agent and this drug, too, proved embryotoxic in the sea urchin model, likely reflecting its interference with catecholamine mechanisms required for embryo assembly [8,11].

Like the central nervous system in mammals, the sea urchin embryo possesses significant diffusion barriers that limit the access of exogenous chemicals to their various targets within the organism. In our earlier studies with agents protecting the embryo from toxicant injury, we readily distinguished the importance of lipophilicity [7,11-14,19], as reinforced here by the descending potency of 5HT derivatives when progressing from AA-5HT to 5HT to 5HTQ. Nevertheless, this raises an interesting question about both A β 42 and APP₉₆₋₁₁₀: since both are peptides, how do these manage to enter? Recent publication of the sea urchin genome reveals a number of sequences encoding APP₉₆₋₁₁₀- or amyloid-binding proteins [39], and it would be worthwhile to examine whether these represent transporters or cell surface receptors that initiate endocytotic uptake of APP₉₆₋₁₁₀ and/or A β 42. Alternatively, these agents may be far more effective than implicated by their nominal concentration in the medium, so that penetration of even a small proportion of the molecules may be sufficient to elicit an effect.

Our findings have potential implications for mechanisms of neurodevelopmental disorders as well as for the development of therapies to offset the actions of neurotoxicants. Polymorphisms in APP are known to be associated with increased risk of neurodegenerative disease [25], and the sea urchin model could clearly contribute to the rapid evaluation of how different mutations influence the trophic effectiveness of the protein and its potential role in neurodevelopmental disorders. Second, we found that a variety of neurotransmitter-related agents centering around ACh, 5HT and cannabinoids, all had beneficial effects in offsetting the toxicity associated with A β 42 and APP₉₆₋₁₁₀, and we there was further improvement with cyclic AMP promoters and antioxidants. Thus, a strategy that combines compounds from all these classes might prove useful in offsetting the adverse outcomes of developmental neurotoxicants of different classes. Whereas evaluations of such combinations would require prolonged studies and extensive

resources in mammalian models, the sea urchin embryo can provide an initial, rapid screening tool to identify those combinations most likely to succeed. Outcomes identified using morphology in sea urchin embryos can then guide evaluations at the biochemical and molecular level to identify mechanisms and outcomes that are critical to developing novel approaches to the prevention or amelioration of neurotoxicity. As shown here, the sea urchin embryo provides some of the primary information for establishing a specific participation of APP_{96–110} in the susceptibility to developmental disorders, and most especially for the screening of agents that may prove useful in preventing or offsetting developmental neurotoxicity.

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Abbreviations

5HT, 5-hydroxytryptamine, serotonin; 5HTQ, N,N,N,-trimethyl 5HTAA-5HT, arachidonoyl serotonin (arachidonoyl 5-hydroxytryptamine); AA-DMAE, arachidonoyl dimethylaminoethanol; AA-VAN, arachidonoyl vanillylamine; ACh, acetylcholine; β -amyloid 1–42, A β 42; APP_{96–110}, amyloid precursor protein 96–110; ASW, artificial sea water; IBMX, 3-isobutyl-1-methylxanthine.

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Figure 1.

Comparative effects of Aβ42 and APP₉₆₋₁₁₀ on sea urchin embryonic development at different stages: (A) added at the 2–4 cell stage; (B) added at the mid-blastula-2 stage; (C) added at the late gastrula-1 stage; (D) added at the early pluteus stage; the control for B and C is the same, since these were evaluated at the same stage. The durations listed for each condition correspond to the time post-fertilization. Note the greater sensitivity to Aβ42 (lower concentrations required for anomalies) and the correspondingly wider window of vulnerability as compared to APP₉₆₋₁₁₀.

Figure 2.

Protective effect of 5HT analogs (10 µM) against the actions of APP₉₆₋₁₁₀ (2 µM). Test agents were introduced 5–10 min before adding APP₉₆₋₁₁₀ at the mid-blastula-2 stage (10 hr 45 min after fertilization), and evaluations were made at 36 hr 5 min after fertilization. The lipophilic analog, AA-5HT, provides partial protection whereas the hydrophilic analog, 5HTQ, is ineffective.

Figure 3.

Comparative protection by AA-5HT and nicotine against Aβ42 (top) and APP₉₆₋₁₁₀ (bottom). Test agents were introduced 5–10 min before adding Aβ42 or APP₉₆₋₁₁₀ at the mid-blastula-2 to late blastula-1 stage (13 hr after fertilization). Evaluations were made at the early gastrula-2 stage (18 hr) for Aβ42, after which the unprotected larvae died. At the same stage, APP₉₆₋₁₁₀-treated embryos looked essentially the same as the Aβ42 group (not shown), and subsequent evaluations were made at the early pluteus stage (36 hr 50 min) shown here. Note the effective protection by either AA-5HT or nicotine against both Aβ42 and APP₉₆₋₁₁₀.

Figure 4.

Protective effects of different test substances (20 μ M) against the actions of APP₉₆₋₁₁₀ (2 μ M). Test agents were introduced 5–10 min before adding APP₉₆₋₁₁₀ at the mid-blastula-2 to late blastula-1 stage (13 hr after fertilization, first control picture), and evaluations were made at the early pluteus stage (36 hr 50 min after fertilization, second control picture). Note the arrested development and malformation evident from APP₉₆₋₁₁₀ exposure alone. Nicotine and tocopherol provide the best protection, whereas IBMX, caffeine and physostigmine are less effective, and d-tubocurarine the least effective. The AA-5HT and nicotine pictures are from the same samples shown in Fig. 3.

Summary of protective effects of different classes of agents on the sensitivity of developing sea urchins to embryotoxicants

TABLE 1

Target	Agent	Toxicant			
		Aβ42	APP _{9c-10}	Chlorpyrifos	Reserpine
ACh	AA-DMAE	+++ or ++	+++	+++	++
	Nicotine	++	+++	0	+
	d-Tubocurarine	+	+	+	+
	Physostigmine	++	++	NT	NT
5HT	Tacrine	Tox	Tox	NT	NT
	AA-5HT	+++	++	++	+++
	5HT	+/-	+/-	+/- or 0	+
	5HTQ	+/- or 0	0	0	0
Cannabinoid/vanilloid	AA-VAN	++ or +	++ or +	+	++
NMDA	Memantine	Tox	Tox	NT	NT
α-adrenergic	Nicergoline	Tox	Tox	NT	NT
cAMP	Caffeine	++	++	NT	NT
	IBMX	NT	+++	++	NT
	α-Tocopherol	++	+++	NT	NT

+++ strong protection, up to complete normalization of development

++ strong but incomplete protection

+ moderate but obvious protection

+/- marginal protection

0 no protection

Tox protective agent was itself toxic to embryos

NT not tested

Results for chlorpyrifos and reserpine are taken from earlier publications with this model [7,11–14,19]. Where there are double entries (e.g. ++ or +), the outcome varies with the stage of development tested.