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EFFECTS OF D-α-AMINOADIPATE ON EXCITATION OF AFFERENT FIBERS IN THE LATERAL LINE OF *XENOPUS LAEVIS*

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The effects of D-α-aminoadipate (DαAA) on excitation of afferent nerve fibers in the *Xenopus laevis* lateral line were studied in vitro. DαAA reversibly suppressed spontaneous activity and excitation induced by water motion at concentrations as low as 0.25–0.5 mM. Higher concentrations (up to 10 mM) caused a greater suppression that was rapidly and fully reversible. L-α-Aminoadipate at 0.25–1.0 mM had no suppressive effects. Responses elicited by NMDA (1.0–2.0 mM) were the most sensitive to DαAA (0.25–0.5 mM), those elicited by L-aspartate and L-glutamate (1.0–2.0 mM) were less sensitive and similar, and those elicited by kainate (5–15 μM) were the least sensitive. The results provide evidence that the transmitter released by hair cells in the *Xenopus* lateral line interacts postsynaptically with NMDA-preferring receptors and that the transmitter is an excitatory amino acid, possibly L-glutamate or L-aspartate.

The identity of the afferent transmitter released by hair cells in acousticolateralis organs, including the amphibian lateral line, is not known, though evidence suggests that it is an excitatory amino acid [3, 4, 6, 10, 11]. One of the criteria for identifying a transmitter substance is to show that pharmacological antagonists which block the postsynaptic response induced by natural stimulation also block the response induced by the exogenously applied proposed transmitter. In the mammalian and amphibian central nervous systems (CNS), one of the most extensively studied excitatory amino acid antagonists is D-α-aminoadipate (DαAA). It is thought to block L-glutamate and L-aspartate in a competitive manner and without influencing postsynaptic membrane potentials [1, 2, 12, 13]. The present study was undertaken to investigate the actions of DαAA on afferent activity in the *Xenopus* lateral line. Its effects on spontaneous and water motion-induced activity as well as on responses

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to exogenously applied N-methyl-D-aspartate (NMDA), L-glutamate, L-aspartate and kainate were evaluated.

Experiments were performed on male and female African clawed frogs (*Xenopus laevis*). The methods for anesthetizing the animals, removing a portion of skin containing the lateral-line organ, and isolating a single stitch (cluster of hair cells) were as previously described [3, 5]. Action potentials from the two afferent nerve fibers that innervate the stitch were recorded extracellularly with a suction electrode, digitized and counted with a rate-meter in 1-min time bins [3, 5].

To evaluate the antagonist activity of DαAA on water motion-induced responses, skins containing the lateral line were held on the end of a plastic cylinder with a Neoprene O-ring [3]. Vibrating water motion was applied tangentially as previously described [3] to the external (cupular) surface of the skin which faced down into a water-filled chamber. Stimulus magnitude was adjusted to elicit a submaximal response in each experiment. All preparations were continuously perfused at 2 ml/min with a drug-free frog Ringer solution [3] at room temperature except during periods of testing. For testing, 3 min of spontaneous and 3 min of stimulated activity were alternately recorded 5–6 times, in the absence and presence of DαAA in 400 μl of Ringer solution placed in the experimental chamber to cover the inner (serosal) surface of the skin. Mean spontaneous and stimulated firing rates were calculated from the second and third minutes in each of the last 4 samples of spontaneous and stimulated activity. These values, as well as the difference between them, in the presence of DαAA were expressed as a percentage of those obtained in its absence.

The preparation described by Bobbin et al. [5] was used to study the effects of DαAA on responses to agonists applied to the serosal surface. Responses to excitatory amino acids were obtained during an initial drug-free Ringer wash, 50 min after starting a wash containing DαAA and again 50 min after washing the preparation with the drug-free Ringer solution. For each response, 5 min of spontaneous activity were recorded, then the respective wash was turned off one minute before drug application. Excitants were applied to the stitch in a 50 μl volume of Ringer solution and remained in contact with it for 5 min at which time the respective Ringer wash was turned back on. The activity before drug application was used to calculate a mean spontaneous firing rate. The difference in the peak excitation elicited by an excitant and the pre-drug spontaneous level in the presence of DαAA was expressed as a percentage of that obtained in its absence. Drugs were dissolved in the Ringer solution [3]. The L-glutamate, L-aspartate, kainate, D-α-aminoadipate, and L-α-aminoadipate were purchased from Sigma (U.S.A.) and the N-methyl-D-aspartate was purchased from Tocris (U.K.).

The antagonistic effects of DαAA (0.25 mM) on spontaneous activity and water motion-induced excitation of lateral line afferent fibers are illustrated in Fig. 1. In this preparation 0.25 mM DαAA suppressed spontaneous activity 20% and decreased the absolute stimulated firing rate 30%. The difference between the mean spontaneous and stimulated firing rates was suppressed 50% in the presence of DαAA. The

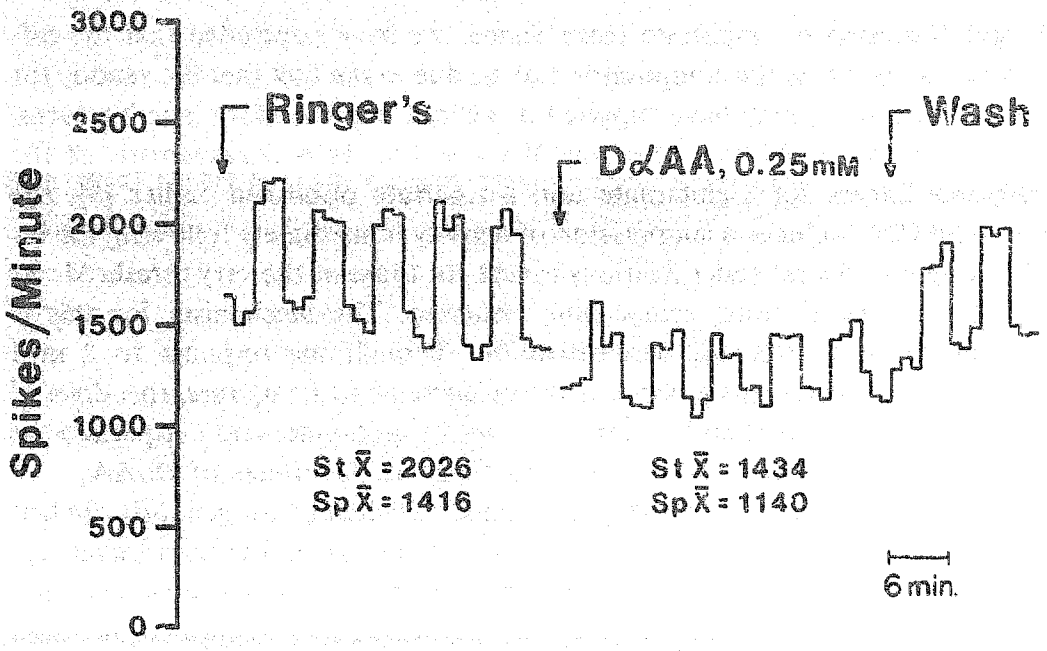

Fig. 1. Suppression of spontaneous activity and water motion-induced excitation of afferent nerve fibers in the *Xenopus* lateral line by 0.25 mM DαAA. At the arrow designated Ringer's the drug-free Ringer wash was turned off and the remaining fluid on the serosal surface of the skin rapidly exchanged for a 400 μl volume of fresh drug-free Ringer's. An initial 3 min of spontaneous activity followed by five 3-min periods of stimulated and spontaneous activity were recorded. At the arrow designated DαAA, the 400 μl of Ringer's in the chamber was rapidly exchanged for 400 μl of Ringer's containing 0.25 mM DαAA. The stimulated–spontaneous sampling sequence was then repeated following a 2 min break in the recording and at the arrow designated wash, the drug-free Ringer wash was turned back on and the preparation permitted to recover. Mean values for the stimulated (St) and spontaneous (Sp) conditions obtained in each of the solutions are given and were calculated as described in the text.

effects of DαAA were typically rapid in onset with both spontaneous and stimulated activity recovering quickly and completely in the drug-free Ringer wash (Fig. 1). In one experiment, following marked suppression induced by 10 mM DαAA, both spontaneous and stimulated activity recovered to pre-drug levels within 12–15 min after commencing the Ringer wash. In general, higher concentrations of DαAA caused greater suppression of both spontaneous activity and water motion-induced excitation. The effects of L-α-aminoadipate (LαAA) were studied in 3 experiments and found to produce no marked change in lateral-line activity at 0.5 mM, while at 1.0 mM there was an increase in both stimulated and spontaneous activity.

In contrast to the negative results with NMDA reported earlier [5], experiments with NMDA (n = 3) from a different source (Tocris) have revealed the Tocris NMDA to be approximately 0.5–2 times as potent as L-glutamate in producing an increase in spike rate. The NMDA used previously was confirmed as inactive by comparing it to the Tocris NMDA on the same preparation. By comparing high pressure liquid chromatograms of dansylation derivatives of both NMDA com-

pounds and N-methyl-D,L-aspartate from Sigma, we have concluded that the difference observed between the compounds may be due to the fact that the vendor for the inactive NMDA [5] may have supplied a cyclized and therefore inactive form, an error initially described by Curtis and Watkins [7]. As is characteristic of the dose–response curves for L-glutamate and L-aspartate published earlier [5], the response to NMDA includes a suppression of activity immediately following the increase in firing rate for all concentrations except for those at the very threshold. At threshold the increased firing rate is not sustained. The mechanism for this is unknown but may be receptor desensitization. Though the response to 2 mM NMDA consistently produced a post-excitatory decrease in firing rate, this dose of NMDA was equivalent or slightly greater to 1 mM L-glutamate and L-aspartate, so it was chosen to stringently illustrate the specificity of the effects of DαAA.

The effects of DαAA (0.25–1.0 mM) on responses elicited by exogenously applied amino acids were evaluated in 7 experiments which met several criteria including: a stable spontaneous rate greater than 400 spikes per minute, a detectable response to 2 mM L-glutamate, and a final response to L-glutamate that duplicated the intial one. In the presence of DαAA (0.25–0.5 mM) agonist-induced excitation was selectively affected, with sensitivity to DαAA ranked as: NMDA > L-aspartate = L-glutamate > kainate. In two preparations, 0.25 mM DαAA reduced spontaneous activity (24%), abolished the excitatory response to 1.0 mM NMDA (n = 1), reduced (53%) the response to 2.0 mM NMDA (n = 1), had little (< 10%) effect on responses to 1 mM L-aspartate (n = 1), and 1 mM L-glutamate (n = 2) and increased (16%) the response to 5 μM kainate (n = 1). Results from one of these experiments are illustrated in Fig. 2B. In 4 preparations 0.5 mM DαAA abolished (88%, range 77–95%) the response to 2 mM NMDA (n = 3), reduced the response to 1 mM L-glutamate (n = 4) and 1 mM L-aspartate (n = 3) equally (22%, range 11–40% and 25%, range 20–33%, respectively), increased (26%) the response to 5 μM kainate (n = 2), and reduced (56%, range 30–75%) spontaneous activity (n = 4). Results from one of these experiments are depicted in Fig. 2C. In one preparation 1 mM DαAA abolished spontaneous activity and the responses to 2 mM L-glutamate, 2 mM NMDA and 15 μM kainate.

The results show that DαAA, but not LαAA, reversibly suppresses spontaneous and stimulus-induced activity of lateral-line afferent fibers. In the CNS it has been shown that at the concentrations (0.5–1.0 mM) used in the present study, DαAA selectively blocks excitatory amino acid receptors with little if any effect on receptors for acetylcholine, catecholamines or substance P [3]. Therefore, it has generally been maintained that suppression of synaptic activity by DαAA indicates the transmitter is an excitatory amino acid. At least 3 types of receptors for excitatory amino acids may exist in the mammalian and amphibian CNS [14], with the non-endogenous compounds NMDA and kainate purported to be selective agonists for two of these receptor types. We have shown that the response to NMDA is most sensitive to blockade by DαAA at concentrations that reduce water motion-induced

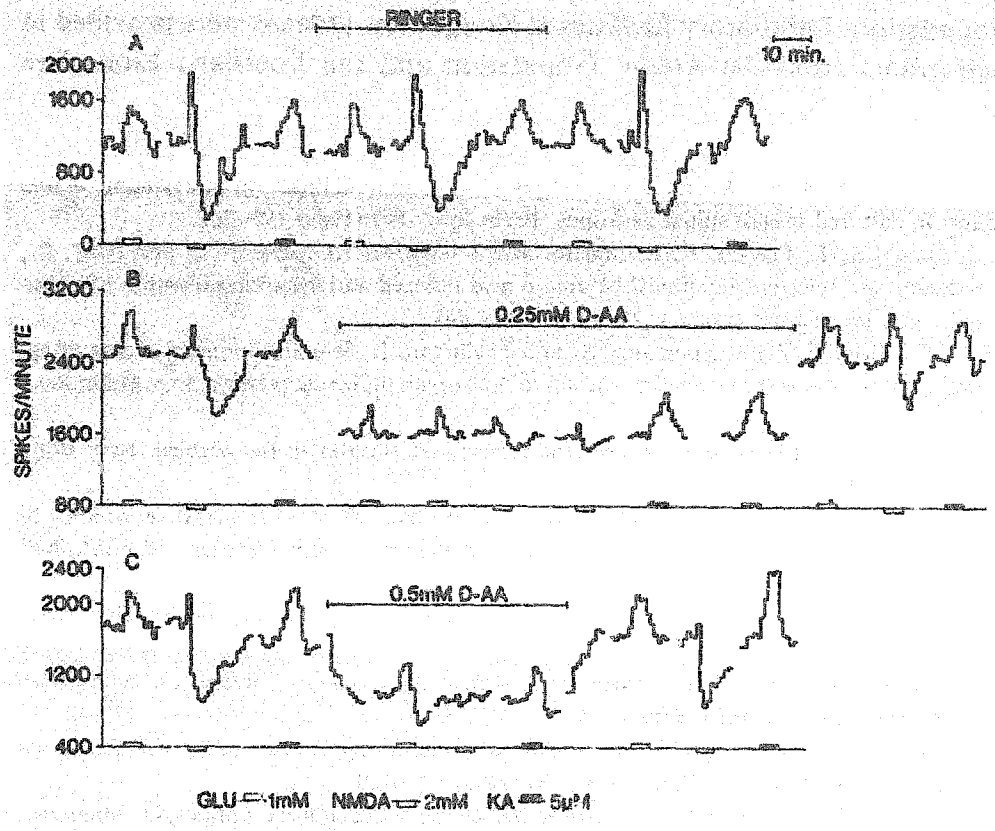

Fig. 2. Responses of lateral line afferent fibers to exogenously applied L-glutamate (GLU), N-methyl-D-aspartate (NMDA) and kainate (KA) in the presence of drug-free Ringer solution (A), 0.25 mM DαAA (B) and 0.5 mM DαAA (C). Results are from 3 different experiments. Open and closed bars indicate agonist concentrations and the time of contact with the preparation.

stimulation, thus it follows that the transmitter released from hair cells may be an excitatory amino acid acting at NMDA receptors. DαAA did not affect the response to kainate at concentrations that suppressed spontaneous activity and blocked NMDA, thus it seems possible that DαAA specifically blocked receptors that mediate both spontaneous activity and the NMDA response. Since L-aspartate and L-glutamate appear to be equally suppressed by DαAA, no evidence was obtained as to which natural substance is preferred at these receptors. The actions of DαAA on lateral-line activity are in contrast to a recent report showing its lack of effect in the mammalian cochlea [9]. This would suggest that, at present, assumptions concerning the similarity of synaptic chemistry among the various types of acousticolateralis organs should be made with caution.

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