Gustatory Response in the Puffer—III Stimulatory Effectiveness of Nucleotides and their Derivatives

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The stimulatory effects of nucleotides and related compounds on the lip chemoreceptors of the puffer, *Fugu pardalis*, were studied by recording electrical responses from the facial nerve innervating the upper lip.

AMP, ADP, ATP, IMP and UMP elicited a response in the nerve, while other ribonucleotides, CMP, GMP and XMP, and deoxyribonucleotides, dAMP, dCMP, dGMP and TMP, did not at 10^{-5} M. Studies with single-fiber preparations showed that all of the above effective nucleotides tend to stimulate the same taste units.

While AMP at 10^{-5} M had a marked stimulatory effect, its component compounds, adenine, D-ribose, inorganic phosphate, adenosine and D-ribose-5-phosphate at the same concentration, all failed to elicit any detectable responses. Adenosine-3'-monophosphate and adenosine-3', 5'-monophosphate at 10^{-5} M also did not elicit any appreciable responses.

A sample of RNA from yeast was also tested. It did not appear to be effective.

It has previously been found that the lip chemoreceptors of the puffer, *Fugu pardalis*, respond well to adenosine-5'-monophosphate (AMP) and some other ribonucleotides.¹⁾ In this study, several different nucleotides and related compounds were further examined for their stimulatory effects on this fish.

Materials and Methods

A total of 43 puffers, *Fugu pardalis*, weighing 70 to 320 g was used. The procedure for obtaining the facial nerve (ramus maxillaris²⁾) preparation and the recording systems for the integrated response³⁾ of the whole nerve and impulse discharge of the single fiber were principally the same as those described in previous reports of this series.^{1,4)} Some modifications were made in the present study of the method of stimulus application. Using a three-way tube with a stopcock, sea water and test solutions were applied in succession: A steady gentle stream of sea water was first allowed to flow on to the lip through one of the two inlet openings; then the stopcock was turned quickly to allow the test solution to flow through. This successive application of sea water and test solutions enabled the test solutions to touch the lip surface with little mechanical stimulation, as will be illustrated below. The stimulants used were dissolved in sea water unless otherwise stated. All of the nucleotides and related chemicals were obtained commercially. The experiments were performed during the period from January to May.

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Fig. 1. Neural responses recorded from the ramus maxillaris on application of sea water and 10^{-8} M solutions of nucleotides and related compounds to the upper lip of the puffer. Upper trace: the integrated response, lower trace: the direct response. A and B are from different preparations. See text for details.

Results and Discussion

Fig. 1 shows an example of the recorded responses from the whole nerve preparation. In each recording in the figure, the upper trace shows the integrated response and the lower trace shows the multifiber activity which produced the integrated response. In most of the integrated responses, a small phasic response is noted starting at the point indicated by the arrow. This response was elicited by the pressure change caused by turning the stopcock on the test solution in the way described above. For some stimulants in the figure, for example AMP, a second rise of response follows within a second after the first one, indicating the arrival of the solution upon the lip. Since almost no appreciable responses are correspondingly yielded to the other solutions in the figure, for example sea water, it is seen that this second response is almost solely due to chemical stimulation. Thus, the results in the figure show that while 10^{-8} M solutions of AMP, adenosine-5'-diphosphate (ADP), adenosine-5'-triphosphate (ATP), inosine-5'-monophosphate (IMP) and uridine-5'-monophosphate (UMP) had a marked stimulatory effect on the lip chemoreceptors, other ribonucleotides, cytidine-5'-monophosphate (CMP) and guanosine-5'-monophosphate (GMP) of the same strength did not stimulate the chemoreceptors. This was true also with the two other preparations investigated. Another ribonucleotide, xanthosine-5'-monophosphate (XMP), and deoxyribonucleotides, 2'-deoxyadenosine-5'-monophosphate (dAMP), 2'-deoxycytidine-5'-monophosphate (dCMP), 2'-deoxyguanosine-5'-monophosphate (dGMP) and thymidine-5'-monophosphate (TMP) were also tested at 10^{-8} M, but no appreciable responses were observed in the one or two specimens used.

In previous experiments,¹⁾ it was noted that ADP, IMP and UMP tended to elicit a response in the same nerve fibers. In the present study, experiments on the single fiber were further conducted including AMP and ATP as stimuli. A total of 19 fibers which

Fig. 2. Responses of two fibers to nucleotides and other taste solutions. Data for the fiber with larger spikes are given in Fig. 3 as fiber J.

Fig. 3. Histogram showing the number of impulses in the responses of the 19 nucleotide-sensitive fibers in the first 2 sec after application of the stimulus. Stimulus solutions in left column, from top, 10^{-3} M AMP, ADP, ATP, IMP and UMP; those in right column, 10^{-2} M L-alanine, glycine, L-proline, betaine and quinine (in distilled water), 5×10^{-3} M HCl and 1.5 M NaCl (in distilled water).

responded to at least one nucleotide were obtained from 15 animals. A typical example of their responses to nucleotides and other stimulants is shown in Fig. 2. It is seen in the figure that the fiber with larger spikes tended to respond uniformly to AMP, ADP, ATP, IMP and UMP. This was true of the majority of the other nucleotide-sensitive fibers. Fig. 3 summarizes the impulse numbers of all the 19 nucleotide-sensitive fibers in response to 10^{-3} M AMP, ADP, ATP, IMP and UMP, 10^{-2} M L-alanine, glycine, L-proline, betaine and quinine hydrochloride (in distilled water), 5×10^{-3} M HCl (pH 2.5) and 1.5 M NaCl (in distilled water) during the first two seconds after the application of the stimulus. Most of the fibers responded to AMP, ADP, ATP and UMP; fibers responding well to one of these nucleotides tended to respond also well to the others. The majority of the fibers in the figure responded also to IMP, but some fibers did not do so. Whether this indicates that different receptors were involved with IMP or means only that the threshold of IMP was high in comparison with those of the other nucleotides is not clear at present. In such animals having fibers not responsive to 10^{-3} M IMP, only weak responses were elicited from the whole nerve trunk also, whereas in many other animals, 10^{-3} M IMP elicited a considerable response (cf. Fig. 1). Quite in the same way as observed previously¹¹ in fibers responding to ADP, IMP and UMP, most of the nucleotide-sensitive fibers in Fig. 3 did not respond to alanine, glycine, proline, betaine and HCl, which were effective on other fibers conversely not sensitive to any of the five nucleotides used here.

In order to study which of the three component parts of AMP (the purine base adenine, ribose and phosphate group) is responsible for the stimulation of the receptor, the effects of adenine, ribose, sodium phosphate, adenosine and ribose-5-phosphate were examined with whole-nerve preparations. An example of the experiments can be seen in Fig. 1. In the figure, no appreciable response is elicited by any of the 10^{-3} M solutions of them, while 10^{-3} M AMP elicits a large response. Mixed solutions of adenine, ribose and sodium phosphate, containing each at 10^{-3} M, and of adenosine and the phosphate, containing each at 10^{-3} M, were also tested, no appreciable responses being yielded. Adenosine-3'-monophosphate (3'-AMP) and adenosine-3',5'-monophosphate (3',5'-AMP) were also tested. Again, no appreciable responses were observed, as also seen in Fig. 1. Thus, these results suggest that the combined structure of all of the three component parts that make up AMP is the stimulus unit. UMP differs from AMP in that the base adenine is replaced by uracil—different in size and with different radicals. Therefore, it is conceivably possible that UMP somewhat differs from AMP in its way of receptor stimulation, or in the receptor groups it stimulates, in spite of the fact that it elicited a response in the same fibers as AMP did (Fig. 3). This was actually the case when the responses to AMP and UMP were differently affected by low pHs, as will be described below. The results in Figs. 1, 2 and 3 suggest that a further binding of phosphate group to AMP

Fig. 4.

Fig. 4. Magnitudes of integrated responses of the whole nerve to various concentrations of AMP, ADP and ATP, plotted in arbitrary units. The pH of each test solution was adjusted to pH 8.0 with HCl and NaOH.

Fig. 5.

Fig. 5. Effects of pH on the responses to AMP and UMP. Ordinate, magnitude of the integrated response of the whole nerve; abscissa, pH of AMP or UMP solution. Low pH solutions were made by adding HCl. Numeral appended to each point indicates the order of the application of the test solutions.

and lower concentrations, but with increasing concentration above 10^{-8} M the effectiveness of each of the three approached the same magnitude, as shown in Fig. 4. A sample of sodium salt of RNA (Kohjin Co.; average molecular weight per mole of P, 344.6 as sodium salt; base molar ratio, AMP: CMP: GMP: UMP = 1.00: 0.75: 1.05: 0.98) was also tested by dissolving 50 mg of it in 100 ml of sea water. No detectable response was obtained.

In the course of preliminary experiments with high concentrations of nucleotides, it was observed that the responses to some nucleotides were suppressed by low pHs caused by their phosphate group. A typical example of the suppressing effect on the response to 10^{-3} M AMP in 0.5 M NaCl is shown in Fig. 5. It is seen in the figure that the response to it was deeply suppressed with decreasing pH from around 6 to near 4, whereas that to 10^{-3} M UMP in 0.5 M NaCl was not significantly affected over the same pH range, indicating that the relative stimulatory effectiveness between the two differs according to the pH of the stimulus solution. The response to ADP was also found to be suppressed by low pH.

Some of the effective nucleotides in the above experiments have also been known to stimulate the taste receptors of mammals.⁵⁻⁷⁾ Some have an acceptable taste for humans and the flavour is markedly enhanced when a minute quantity of monosodium glutamate (MSG) is added.⁵⁾ In the rat and the cat, the addition of some nucleotides to MSG also yielded an enhancement of the neural response of the taste nerve; the response to the alters its stimulatory effectiveness. ADP was more effective than AMP, or ATP, at 10^{-3} M

mixture was much larger in magnitude than the algebraic sum of those to the single solutions of MSG and the nucleotide.^{6,7)} Therefore, another experiment was performed to examine the effects of mixed solutions of MSG and AMP or UMP on the puffer. No detectable change in the magnitude of the integrated whole nerve response was yielded by adding MSG at 10^{-3} M to 10^{-3} M AMP or 5×10^{-4} M UMP in the two preparations used (MSG itself was not effective up to 10^{-2} M). Experiments were further conducted using 19 other amino acids and betaine in place of MSG with one to three preparations for each; again, no noticeable modification of the response to AMP or UMP was observed; on the other hand, there was an enhancement of the response between pairs of some of the amino acids plus betaine, about which, a report will be given elsewhere.⁸⁾

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