

inhibitory vagal reflex is accentuated, which is in agreement with the early observation of Ngai⁶. However, we should point out that our approach may lead to somewhat different conclusions from those of more usual experiments where the prolongation of T_E during maximal occlusion is taken as the measure of the strength of the BH reflex. An example is the result of Grunstein and Grunstein⁵. These authors concluded that enkephalin, an endogenous opioid peptide, does not change the inspiratory-inhibitory vagal reflex in the newborn. However, if the reflex strength is expressed as the ratio of T_E (occlusion) to T_E (control), their data show that the reflex strength decreases. It seems, therefore, that the ontogenesis of opiate modulation of the vagal respiratory input may require a closer investigation.

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Off-responses of newt pit organs after chemical stimulation¹

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Summary. Distilled water rinsing after stimulation of the newt pit organ with divalent chloride salts produced neural off-responses. The off-response was selectively depressed by $HgCl_2$ treatment and was not suppressed by a water rinse containing $NaCl$. The results indicate that the off-response might be due to the removal of the divalent cations bound to the receptor membrane.

Key words. Newts; *Cynops pyrrhogaster*; pit organ; neural off-response; $HgCl_2$ treatment.

Some investigators have demonstrated that in aquatic vertebrates a lateral line organ, the pit organ, has a conspicuous sensitivity to various chemical stimuli³⁻⁶. Previously we reported that when the newt lower jaw, on which many pit organs are distributed, was rinsed with distilled water. After a chemical stimulation, a marked integrated response was obtained from a mandibular branch of the lateral line nerve supplying the pit organs⁷. There have been no studies on this kind of response in the pit organs, although a similar response, the so-called 'water response', in the gustatory organ has been studied by many investigators⁸⁻¹¹. The aim of the present experiments was to elucidate the effect of a water rinse after stimulation of the newt pit organ with divalent chloride salts.

Materials and methods. Male newts (*Cynops pyrrhogaster*) anesthetized with urethan (9 mg/g b.wt, i.p.) were used in the present experiments. Impulse discharges recorded from a mandibular branch of the lateral line nerve were passed through an electronic integrator circuit (Nihon Kohden Model EI-600G) with a time constant of 0.3 s and displayed on an ink-writing recorder. Test solutions made from reagent grade chemicals dissolved in distilled water were applied to the lower jaw at the flow rate of approximate 6 ml/min, after that distilled water was delivered continuously to rinse the lower jaw at the same flow rate. The response produced by the distilled water rinse is here termed the 'off-response' against the 'on-response' produced by the test solution. The height in mm, from the level immediately before the onset of each of the integrated on- and off-responses to the peak, was used as their response measures (see inset in fig. 2). To examine the effects of pretreatment with transition metal ion on the off-response, in some cases test solution was delivered after the lower jaw was treated with $HgCl_2$ or $CuCl_2$ for 1 min. After the treatment the lower jaw was rinsed continuously until immediately before the application of test solution. The effects of chelating agents after the treatment were also examined. The room temperature was kept at $20 \pm 2^\circ C$ throughout the experiment. All the solutions and

distilled water used were adapted to room temperature prior to use.

Results and discussion. Figure 1 represents the relation between the off-response of the newt lateral line nerve after $CaCl_2$ stimulation and $CaCl_2$ concentration. Threshold concentration for the off-response after $CaCl_2$ was around 10^{-5} M. The magnitude of the off-response increased with increasing concentration of $CaCl_2$ at lower concentrations, but it decreased abruptly at higher concentrations above 0.3 M (fig. 1). This type of relationship between off-response and concentration was common to other divalent chloride salts, $SrCl_2$ and $MgCl_2$. The magnitude of the off-response had a tendency to depend on the duration of stimulation by $CaCl_2$; in general, it increased slightly with stimulus duration up to 1 min. However, the magnitude of the off-response was unchanged for successive $CaCl_2$ stimulations with a given stimulus duration. On the other hand, the magnitude of the on-response to $CaCl_2$ increased with the concentration from 10^{-5} M to 3×10^{-2} M, while it decreased gradually above 0.1 M, as described previously⁷. Similar concentration ranges for the production of on- and off-responses were obtained for $SrCl_2$ and $MgCl_2$. The relationship between the magnitude of the on-response to 0.1 M $CaCl_2$, which produced the largest off-response, and that of the subsequent off-response in 21 newts is shown in figure 2. A highly significant negative correlation coefficient was noticed between the two responses ($r = -0.90$, $p < 0.001$ $n = 21$), and the algebraic sum of individual on- and off-responses showed a similar value. A similar relation between on- and off-responses was observed for 0.1 M $SrCl_2$ ($r = -0.92$, $p < 0.001$ $n = 17$) and 0.1 M $MgCl_2$ ($r = -0.92$, $p < 0.001$, $n = 17$).

The relation between the $CaCl_2$ response and the subsequent off-response in the present experiments closely resembles the results¹¹ obtained from the rat chorda tympani nerve, in which water applied to the tongue after stimulation with 1 M sucrose produced an off-response. There existed an inverse relation be-

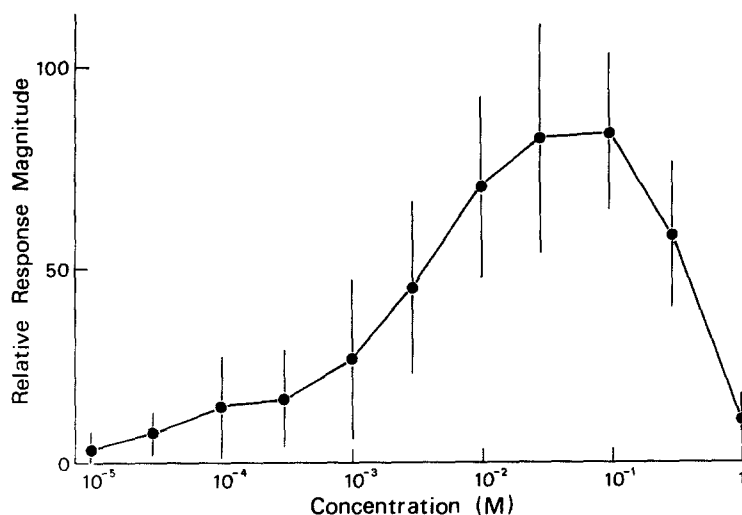


Figure 1. Relative magnitude of off-response of the lateral line nerve for CaCl_2 as a function of CaCl_2 concentration. Each value is expressed relative to the magnitude of the response to 0.1 M NaCl. Vertical lines represent \pm SD of the mean ($n = 5$).

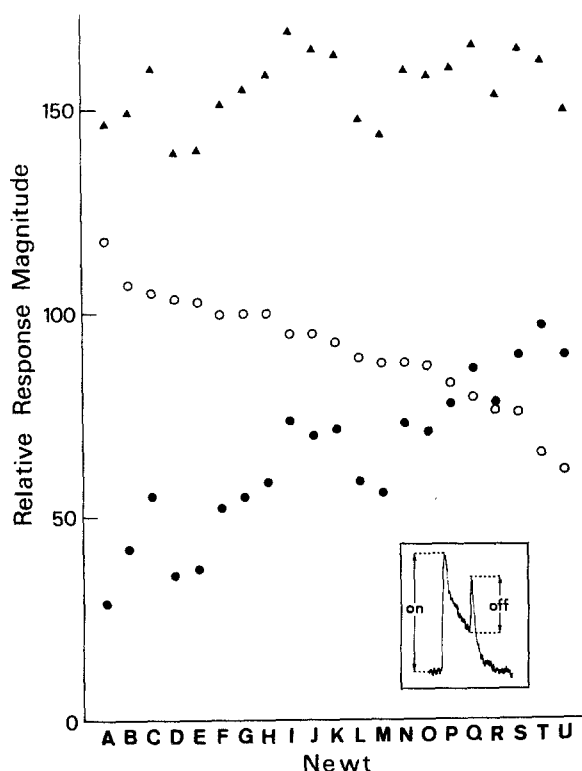


Figure 2. Reverse relation between magnitudes of on- (open circle) and off- (filled circle) responses for CaCl_2 in individual preparations. Each value is expressed relative to the magnitude of the response to 0.1 M NaCl. 21 preparations are arranged in order of the magnitude of the on-response. The algebraic sum (triangle) of magnitudes of on- and off-responses in each preparation differs slightly. Inset shows measures of integrated on- and off-responses.

tween the magnitude of the sucrose response and its corresponding off-response, compared between rats; that is, the larger the on-response, the smaller the off-response. In addition, the algebraic sum of both responses in each rat showed a nearly constant value. According to Yamamoto and Kawamura¹¹, the off-response after sucrose stimulation in the rat is

the water response, which is generally known to be inhibited by adding NaCl to the distilled water.

The present off-response after CaCl_2 was not inhibited appreciably by rinsing with NaCl solutions of concentrations between 10^{-7} and 10^{-2} M (fig. 3). Each of the off-responses produced by various NaCl solutions showed a temporal pattern similar to the off-response produced by the distilled water rinse, as shown in the figure. Therefore, it is unlikely to be a response to water per se. It has been demonstrated that the water response of the frog tongue is extremely diminished by adding NaCl to the water, at concentrations between 10^{-3} M and 10^{-2} M⁹.

A pretreatment of the pit organs with transition metal solutions such as HgCl_2 and CuCl_2 for 1 min affected the off-response after stimulation by divalent chloride salts. Typical on- and off-responses for CaCl_2 before and after treatment with 3×10^{-4} M HgCl_2 for 1 min are shown in figure 4, A and B, respectively. In figure 4, B, the off-response is mostly suppressed after the treatment, while the on-response is not affected or is slightly accelerated. The off-response magnitude was diminished to $19.2 \pm 6.1\%$ (SE, $n = 8$) of that before the treatment and was sustained for over 1 h, but the off-response reappeared and returned readily to the original level after treatment with excess β -mercaptoethanol or dithiothreitol, which are strong chelating agents for the transition metals (fig. 4, C).

This selective and reversible reduction of the off-response indicates that the off-response after CaCl_2 is caused by a mechanism different from the on-response. That is, CaCl_2 may stimulate a group of receptor sites, e.g. by adsorbing or desorbing to them as in the gustatory system^{9,12}, producing an on-response, while it may also inactivate or inhibit temporarily the remaining receptor sites by binding tightly with them; a removal of CaCl_2 from the inactivated receptor sites by the distilled water rinse may induce a conformational change in the cells and cause an off-response. Transition metal ions such as Hg^{2+} and Cu^{2+} probably bind more tightly to the receptor sites than Ca^{2+} and are thus hardly removed by the distilled water rinse without treating with a chelating agent. Since HgCl_2 of low concentration binds selectively to the SH groups of protein and is competitively removed by excess thiol agents such as β -mercaptoethanol and dithiothreitol¹³, it is likely that Ca^{2+} ions may bind, at least in part, to SH group of proteins in the receptor cells.

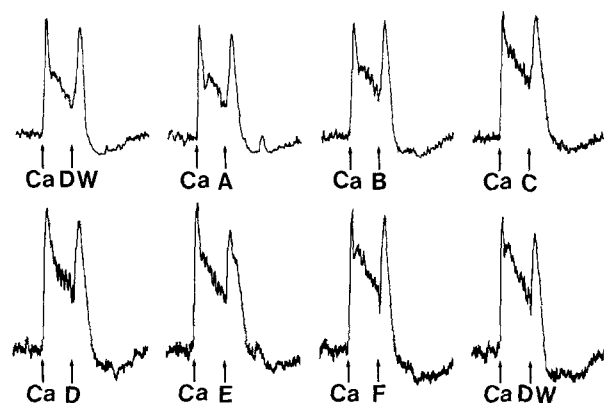


Figure 3. Off-responses produced by rinsing with NaCl of various concentrations (A-F) after 0.01 M CaCl_2 (Ca) stimulation for 10 s in a preparation. The magnitude and the temporal pattern of the off-responses do not change appreciably throughout a large concentration range. A, B, C, D, E and F represent 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} and 10^{-2} M NaCl, respectively. D.W. = distilled water. Each solution was delivered at the time shown by the arrows with respective letters.

The observation of a reciprocal relation between on- and off-responses for CaCl_2 , described above, can be explained provided that in each animal the relative ratio of the receptor sites which are stimulated and those which are inactivated by CaCl_2 of a given concentration differs, whereas the sum of the receptor sites affected is similar.

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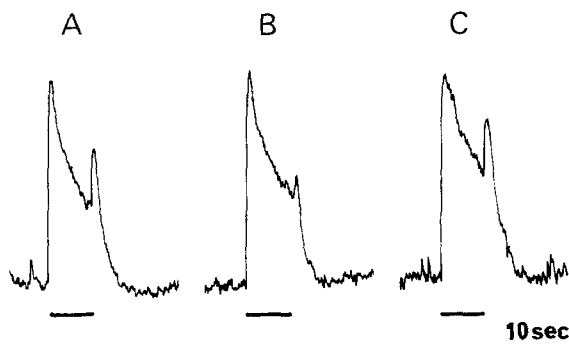


Figure 4. Selective suppression of the integrated off-response after stimulation with CaCl_2 . On- and off-responses before (A) and after (B) the treatment of the pit organs with 3×10^{-4} M HgCl_2 (pH 5.0) for 1 min, and the recovery (C) of the off-response 10 min after the treatment of the organs with 10^{-2} M β -mercaptoethanol (pH 7.5) for 1 min. The bar under each record indicates the duration of the stimulus.

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Blood pressure lowering effect of eicosapentaenoic acid-rich diet in normotensive, hypertensive and hyperlipemic subjects

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Summary. A mackerel diet or a herring diet in which two cans of fish fillet were consumed daily over 2 weeks within a prescribed regimen, in a crossover design, were given to 15 normotensive volunteers, 14 patients with mild essential hypertension and eight patients with type IV and V hyperlipoproteinemia (HLP). In normotensives a markedly lower systolic and diastolic blood pressure at the end of the period on the mackerel diet could be observed, whereas in hypertensive and hyperlipemic subjects only systolic blood pressure was significantly decreased. After the herring diet, which served as control, changes in blood pressure were of a minor degree.

Key words. Eicosapentaenoic acid; mackerel diet; blood pressure; essential hypertension; hyperlipoproteinemia.

Several authors have demonstrated that decreased atherogenic serum lipids and lipoproteins as well as prolonged bleeding time and reduced platelet aggregation follows a high intake of n-3 fatty acids in normal and hyperlipemic subjects¹⁻³. Recently, a blood pressure lowering effect in normotensive volunteers by a diet supplemented with fish oil^{4,5} or canned mackerel⁶ was also described.

The data from healthy men suggested that a favorable effect on blood pressure in patients with arterial hypertension and other diseases accepted as risk factors for atherosclerosis might be

expected. Therefore, we measured blood pressure in normotensive volunteers and in patients with mild essential hypertension as well as with type IV and V hyperlipoproteinemia (HLP) before and after diets supplemented with canned mackerel and herring, respectively, in a commercially available form, which might be more appropriate to nutritional habits in European populations than supplementation with fish oils⁷.

Material and methods. 15 healthy volunteers (10 male, 5 female), 14 male patients with mild essential hypertension (diastolic blood pressure 90-104 mm Hg) and 8 patients (6 male, 2 female)