Effects of barium on separately recorded fast and slow PIII responses in bullfrog retina

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Summary. Investigation of Ba^{2+} effects on fast and slow PIII responses in isolated bullfrog retina revealed that Ba^{2+} suppressed slow PIII completely with little effect on fast PIII. A light-induced $[K^+]_0$ decrease in the photoreceptor layer was observed in spite of Ba^{2+} perfusion, indicating the suppressive action of Ba^{2+} on the K^+ conductance of the Müller cell membrane.

PIII response is composed of fast PIII (receptor potential) and slow PIII. It is well known that the mechanism of fast PIII is due to the sodium conductance decrease of photoreceptor plasma membrane. Concerning the mechanism of slow PIII which originates from Müller cells[2], Witkovsky et al.[3] suggested potassium interaction between photoreceptor and Müller cell. Fujimoto and Tomita[4] successfully reconstructed slow PIII from rod potentials using the analogue circuit proposed by Matsuura et al.[5] which showed the relation between receptor potential and ERG c-wave. Since the c-wave is known to originate in pigment epithelial cells as they hyperpolarize in response to the light-induced decrease in the extracellular potassium concentration, $[K^+]_0$, in the photoreceptor layer, their results positively confirmed that Müller cells also respond to the same change in $[K^+]_0$, giving rise to slow PIII. Matsuura[6] later provided direct evidences to support their view by showing a close correlation in shape and time course between the $[K^+]_0$ decrease and slow PIII.

Barium ion (Ba^{2+}) was shown in frog sartorius fibers to suppress the K^+ conductance[7]. In the retina, using secondary ion spectrometry, Ba^{2+} was found in pigment epithelium[8] and also in the area of outer segments[9]. With intracellular recordings from rods of toad, Brown and Flaming[10] suggested an antagonistic action of Ba^{2+} against Ca^{2+} which plays an important role in the phototransduction mechanism. Bolnick et al.[11] indicated that Ba^{2+} could suppress slow PIII in their observations on the shape and time course of the PIII response in perfused bullfrog retina, and suggested the possibility of a direct action of Ba^{2+} on Müller cells. The present report deals with the separation of fast and slow PIIIs from the PIII response and the investigations of Ba^{2+} effects on the separated 2 potentials. 40 dark-adapted bullfrogs *(Rana catesbiana)* were used. The pigment epithelium was removed from the isolated retina and then the preparation was mounted with receptor side up on small pieces of filter paper, the other side of which was grounded by an Ag-AgCl plate (G). Aspartate-Conway solution as described elsewhere[12] was bubbled with 98% O_2 and 2% CO_2, and perfused continuously to isolate PIII response with a constant flow of 0.5 ml/min through the surface of the receptors. In contrast to the previous work, $MgSO_4$ and Na_2SO_4, which had originally been present at 1.2 and 0.6 mM respectively, were removed to avoid precipitation of Ba^{2+}. Under the conditions of gas bubbling, which started at 1 h before perfusion and continued throughout the experiment, the removal of the phosphates form the original solution had no distinct effect on the pH or on the stability of the response; the pH could be maintained at 7.8 and the PIII response was stable for more than 4 h. To obtain the test solution with Ba^{2+}, 0.01–0.2 mM $BaCl_2$ was simply added to the control solution. Two glass pipette electrodes (1 µm diameter) filled with 5 M LiCl were introduced into the retina; one of them (E_1) was just on the receptor surface and the other (E_2) was in the extracellular space of about 40 µm from the receptor surface. The outputs from 2 unity-gain probes (WPI, M701) were fed into a 2-channel oscilloscope (San-ei, 2317) and then stored in a tape recorder (Teac, R201). With this sort of electrode arrangement, approximately 70% of the fast PIII could be recorded between E_1 and E_2 electrodes. A deeper insertion of E_2 electrode into the retina caused a contamination of slow PIII on the recording because of the anatomical overlapping of the inner segmental region and Müller cell distal portion. Light from a 500-W xenon arc lamp through a 500-nm interference filter and appropriate neutral density filters illuminated the retina vertically; the unattenuated value of light at 500 nm was approximately 40 µV/cm^2.

With increasing light intensity, the total PIII recorded between E_1 and G begins to have a kink, as shown on the top trace of figure 1. A sharp negative going portion up to the kink does not contain any slow PIII since slow PIII is not evoked at this moment. By adjusting the amplitude of the sharp negative going portion of the $[E_1 - E_2]$ recording to that of the total PIII on reproducing the stored data using an ink-writing recorder (San-ei, 8S), the fast PIII, having a real amplitude and time course, could be estimated (middle trace). The slow PIII was obtained simultaneously with a differential record between the total PIII and amplitude adjusted fast PIII (bottom trace). A smooth and slow development of slow PIII was an indication of a good separation of the potential.

Perfusion experiments were performed to investigate Ba^{2+} effects on separated fast and slow PIIIs. Since the fraction of fast PIII in the total PIII depends not only on the flash intensities but also on the E_2 electrode position, the responses evoked by a light of a fixed intensity were compared under control and test perfusions, and caution was also exercised in fixing the electrode position. But, even when the E_2 electrode position

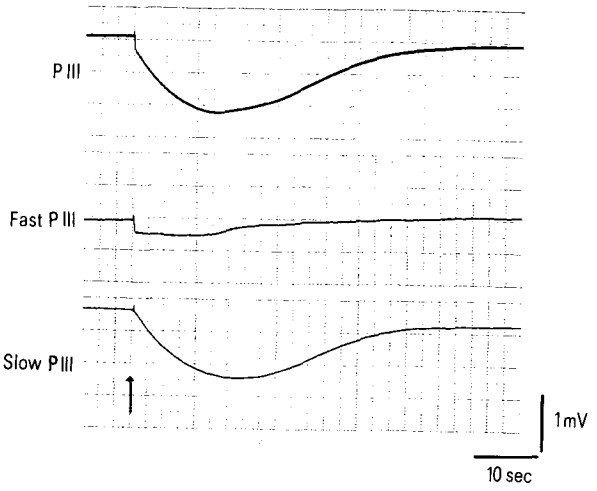

Figure 1. Separation of fast and slow PIIIs from aspartate-isolated PIII response. Two glass pipette electrodes were introduced into the retina, one of which(E_1) was just on the receptor surface and the other (E_2) was in the extracellular space of the photoreceptor layer. Vitreal side of the retina was grounded by an Ag-AgCl plate(G). Top trace: Total PIII response [E_1–G] to a light flash 0.1 sec in duration and −1 log unit in intensity. Note that a sharp negative going potential followed by a slow potential makes a distinct kink. Middle trace: Separated fast PIII. The sharp negative going portion of the [E_1–E_2] recording was adjusted to that of total PIII to estimate the amplitude and time course. Bottom trace: Separated slow PIII obtained simultaneously with a differential record between total PIII and amplitude adjusted fast PIII. The incidence of light is indicated by an arrow mark and can also be recognized by a small positive going artifact on each trace.

was altered to some extent for unknown reasons during perfusion, 2 potentials could be successfully separated by this amplitude adjusting procedure.

As shown in the top traces of figure 2, the total PIII evoked by a light flash 0.1 sec in duration and −2 log units in intensity was separated into fast and slow PIIIs in the control perfusate. The middle traces recorded at 20 min after changing to the test perfusate containing 0.01 mM Ba^{2+}, the minimum concentration in this series of experiments, showed that the shape and time course of the total PIII were drastically changed and that these changes in the total PIII were due to the vanishing of slow PIII; the total PIII in presence of Ba^{2+} was solely composed of fast PIII. The suggestion by Bolnick et al.[11] was firmly substantiated using this kind of separation method. The effects were reversible as shown in the bottom traces recorded at 20 min after returning to the control perfusate. As is clear from the graph in figure 2, Ba^{2+} completely suppressed slow PIII within a few min with little effect on fast PIII, and following return to the control perfusate a rapid recovery of slow PIII was recognized.

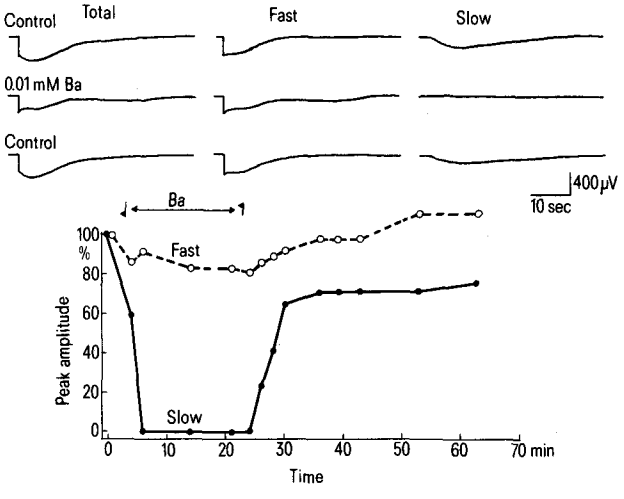

Figure 2. Effects of Ba^{2+} on total, fast and slow PIIIs. Top traces: Recordings in control perfusate. Middle traces: Recordings at 20 min after changing to test perfusate containing 0.01 mM Ba^{2+}. Slow PIII was completely suppressed. Bottom traces: Recordings at 20 min after returning again to control perfusate. The changes in the peak amplitudes of fast and slow PIIIs during Ba^{2+} and control perfusions were shown on the graph. The amplitudes of both potentials recorded just before Ba^{2+} perfusion were taken as 100%. Arrow marks indicate the start and end of Ba^{2+} perfusion. The stimulus was a light flash 0.1 sec in duration and −2 log units in intensity in all cases.

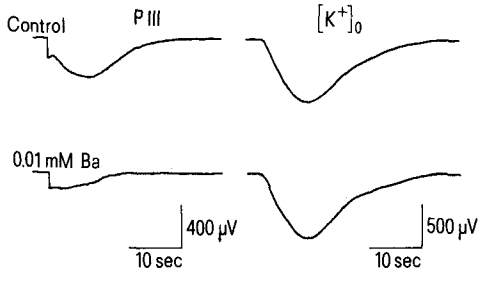

Figure 3. Simultaneous recordings of intraretinal PIII and maximal $[K^+]_0$ change in the photoreceptor layer in control perfusate (upper traces) and in test perfusate containing 0.01 mM Ba^{2+} (lower traces). The stimulus was a light flash 0.1 sec in duration and −1 log unit in intensity. In spite of the slow PIII suppression by Ba^{2+}, the K^+-specific electrode detected the light-induced $[K^+]_0$ decrease which was almost equivalent to that of the control.

It was rather surprising that Ba^{2+} with this low concentration could suppress the slow PIII even in the case where the amplitude was as high as in figure 1. With increasing Ba^{2+} concentration up to 0.2 mM, the maximum concentration used, the effects on both potentials were the same except that the recovery of slow PIII was quite prolonged.

The antagonistic action of Ba^{2+} against Ca^{2+}, that is the increase of the amplitude of fast PIII in presence of Ba^{2+}, was never observed in any of the 18 experiments. The concentration of 0.2 mM may be still too low for Ba^{2+} to exert an antagonistic effect on photoreceptors. The amplitude of fast PIII under Ba^{2+} perfusion was approximately 80–90% in almost all cases and at the most 100% of that of the control. Amplitude decrease to such an extent usually occurred immediately after changing to Ba^{2+} solution and then the fast PIII could be maintained at a steady level for a long period of time.

From the current view that slow PIII is generated by the Müller cell in response to the light-induced $[K^+]_0$ decrease around photoreceptors, there are 2 possibilities to account for the Ba^{2+} suppressive action on slow PIII; one possibility is that Ba^{2+} affects the photoreceptor and the other is a direct action of Ba^{2+} on the Müller cell itself. As already suggested by Bolnick et al.[11] the measurement of $[K^+]_0$ under Ba^{2+} perfusion is essential to ascertain which one is responsible, since the mechanism of $[K^+]_0$ decrease proposed by Matsuura et al.[5] is such that the light stimulus reduces the passive efflux of K^+ out of the photoreceptor with little effect on the active influx of K^+ through the Na^+–K^+ pump in the inner segment. A K^+-specific electrode (5 µm diameter) as described elsewhere[5,6] and a glass pipette electrode, which were set in parallel and close together, with their tips less than 20 µm apart, were inserted into the extracellular space of the photoreceptor layer. The glass pipette electrode served as a reference electrode to the K^+-specific electrode and at the same time as an electrode for simultaneous recording of the intraretinal PIII response. The calibration of K^+-specific electrodes gave an approximate value of 0.2 mM/mV in the vicinity of 3 mM of $[K^+]$. The value was in good agreement with previous work[5,6] and also with the values given by other workers such as Oakley and Green[13].

Although the light-induced $[K^+]_0$ decrease can be observed throughout the photoreceptor layer, its magnitude and peak latency differ depending on the retinal depth. Changing the electrode depth in 5-µm steps, the region giving the maximal $[K^+]_0$ decrease with the minimum peak latency was first determined in the control experiments using weak light stimuli. In agreement with the proposed mechanism[5], the maximal decrease in $[K^+]_0$ was observed around the inner segmental region. The simultaneous recordings of the maximal $[K^+]_0$ change and intraretinal PIII response were then performed using a light flash 0.1 sec in duration and −1 log unit in intensity (fig. 3, upper traces). After changing to the test perfusate containing 0.01 mM Ba^{2+}, the simultaneous recordings (fig. 3, lower traces) were made with precaution concerning the electrode depth, as in the control experiment. In spite of the suppression of slow PIII by Ba^{2+}, the K^+-specific electrode detected the light-induced $[K^+]_0$ change which was almost equivalent to that of the control. In all 10 experiments where Ba^{2+} concentration was varied from 0.01 to 0.2 mM, the decrease in $[K^+]_0$ was always detected. In view of the mechanism of slow PIII, it was concluded from these results that Ba^{2+} suppressed slow PIII by decreasing the K^+ conductance of the Müller cell membrane.

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The cyclic motor activity of the ovine gut: its reset at a faster rhythm

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Summary. In adult sheep, the frequency of the migrating motor complexes (MMC) was increased by the duodenal administration of methysergide, but not affected by other 5-HT_1 or 5-HT_2 antagonists. This reset of the MMC pattern at a faster rhythm suggests a selective action on the enteric neuronal serotoninergic mechanism modulating the pacing of the gut cyclic motor activity.

Recordings of the motor events of the small intestine throughout 24 h indicate that a cyclic activity, the migrating motor or myoelectric complex (MMC), recurs at intervals of 90–120 min during the interdigestive state in dogs[1] or regardless of feeding in ruminants[2]. The existence of an intrinsic mechanism for initiation of the MMC pattern has been located at the duodenal bulb level in sheep[3]. The persistence of MMCs after combined vagotomy and splanchnicectomy, which abolishes the cyclic activity of the stomach (reticulum), indicates the major role of the enteric nervous system[4]. A great variety of substances including serotonin (5-hydroxytryptamine; 5-HT)[5] or substance P[6] have been suggested as enteric neurotransmitters, but none of them are able to increase the daily number of MMCs. Likewise, motilin, pancreatic polypeptide, somatostatin and morphine, which induce premature MMC-like phases of activity, may be considered as triggering agents rather than initiators of the MMC pattern[7]. This report presents the first evidence in both intact and vagotomized sheep of a resetting at a different rhythm of the enteric biological clock, which generates the MMC pattern, by the local administration of 5-HT antagonists.

Materials and methods. 6 ewes, 4 years old and weighing 48–50 kg, were maintained continuously in large cages and prepared for long-term electromyographical and mechanical recording of gastroduodenal motility by serosal implantation of nichrome wires (120 µm in diameter) and fixation of force strain gauges on the duodenum at 3 cm and 23 cm beyond the pylorus and on the proximal jejunum at 1 m from the pylorus[8]. The connecting wires, 2 m in length, were exteriorized in the right flank. In addition, 2 silastic catheters (2 mm in ext. dia.) leading into the lumen of the proximal duodenum and jejunum were inserted and anchored at 3 and 100 cm from the pylorus. An electroencephalograph (Reega VIII, Alvar) set at a paper speed of 30 cm/h was used to register over a period of 3 months the electrical activity of the duodenum and its mechanical activity via a Wheatstone bridge[8].

The animals, fed twice daily (9:00 and 17:00), received through the catheters, within 3 h following the morning meal, either 5-HT_1 or 5-HT_2 antagonists dissolved in a constant volume (5 ml) of distilled water or propylene glycol 20% w/v. The order of treatment (water or propylene glycol; methysergide maleate – courtesy of Sandoz, Basle: 50, 100 and 200 µg/kg; xylamidine tosylate – courtesy of Wellcome, Beckenham: 1 mg/kg and cyproheptadine HCl: 1 mg/kg) was randomized and the timing of injections was normalized by making them at a moment that was a fixed percentage (about 50%) of the previous duration of the MMC in progress. Ketanserin (R 41468) and R 50970 (courtesy of Janssen, Beerse) were used as antag-

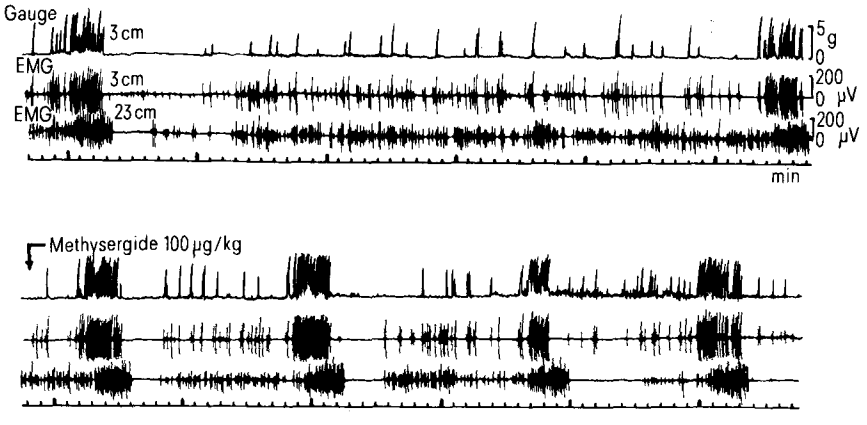

Figure 1. In an intact sheep, electrical (EMG) and mechanical (strain gauge) activity of the duodenal bulb, at 3 cm from the pylorus, showing isolated and in-series contractions lasting about 3 min repeated at 60-min intervals, and propagated to the duodenum, at 23 cm from the pylorus within approximately 1 min. The in-series contractions of the duodenal bulb corresponded to the phases III of the MMC pattern on the proximal duodenum. The intraduodenal administration of methysergide at the dosage of 100 µg/kg reduced for several hours the interval between the in-series contractions of the phases III of the MMC pattern to less than 15 min.