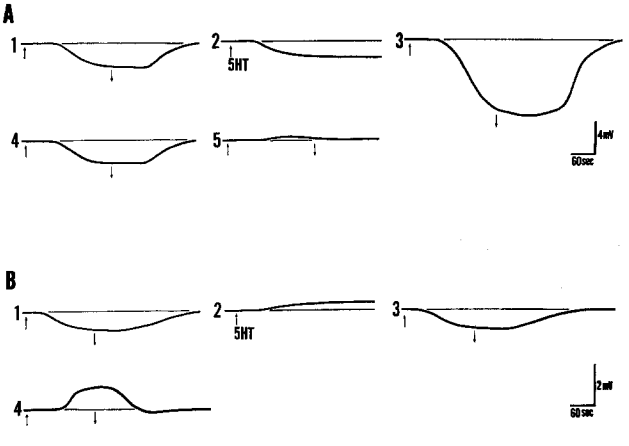
5-HT and the Electrogenic Sodium Pump

The membrane of isolated bullfrog sympathetic ganglion cells in Ringer's solution containing nicotine is hyperpolarized and the slow inhibitory postsynaptic potential (slow IPSP) of these cells is markedly augmented under the effect of 5-hydroxytryptamine (5-HT)[1]. It was postulated that 5-HT accelerated the electrogenic sodium pump[1] by which the slow IPSP might be produced[2].

The present communication reports experimental evidence showing that 5-HT can indeed accelerate the electrogenic Na^+-pump. The K-activated hyperpolarization of bullfrog sympathetic ganglion cells, which is apparently produced by an electrogenic Na^+-pump[3], was markedly augmented under the effect of 5-HT.

Materials and methods. Isolated paravertebral sympathetic ganglion chains and isolated splanchnic nerve trunks of bullfrogs (*Rana catesbeiana*) were used. Changes of the membrane potential in the ganglion cells and in the nerve fibres were measured using the sucrose-gap method[2,3]. The ionic composition (m*M* per 1000 cm^3 H_2O) of the Ringer's solution used was as follows: 112 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$ and 2 m*M* $NaHCO_3$. 2 m*M* KCl was simply omitted in order to provide a K^+-free Ringer's solution. The preparations were continuously superfused with a solution flowing through a chamber ($50 \times 5 \times 4$ mm) at the rate of 0.2 cm^3/sec. The fluid in the chamber could be replaced completely by one of different composition within approximately 5 sec. Compounds added to the solutions were 5-HT creatinine sulfate (Wako), creatinine sulfate (Nakarai), ouabain (Merk) and nicotine sulfate (Katayama).



Effects of 5-HT and ouabain on the K^+-activated hyperpolarization of sympathetic ganglion cells (A) and splanchnic nerve axons (B) of bullfrogs. The preparations were continuously perfused with K^+-free Ringer's solution; the duration of the application of Ringer's solution was shown by arrows (in records 1, 3, 4 and 5 in A), and records 1, 3 and 4 in B)). 0.12 m*M* nicotine was added to the superfusing fluid throughout these experiments. A) Record 1 and record 3 were taken before and at approximately 25 min after an application of 0.1 m*M* 5-HT (record 2), respectively, and record 4 was taken approximately 25 min after withdrawal of 5-HT. 2×10^{-3} m*M* ouabain was added 5 min after record 4, and record 5 was taken 25 min thereafter. Note the hyperpolarization in record 2, and depolarization in record 5. B) Record 1 and record 3 were taken before and approximately 25 min after 1 m*M* 5-HT application (record 2), respectively. 5-HT was withdrawn and 2×10^{-3} m*M* ouabain was added after record 3, and record 4 was taken 20 min thereafter. Note depolarizations in record 2 and record 4.

Results and discussion. The K^+-activated hyperpolarization was first demonstrated with rabbit unmyelinated nerve fibres[3]. A similar K^+-activated hyperpolarization can be observed in bullfrog sympathetic ganglion cells[4]. In the present experiment, isolated sympathetic ganglia were initially superfused with K^+-free Ringer's solution for 60 min. When the fluid was switched to Ringer's solution (containing 2 m*M* KCl), a K^+-activated hyperpolarization of ganglion cells reaching a maximum value (2–4 mV) within approximately 3 min could be recorded (Figure A-1). The maximum amplitude of the K^+-activated hyperpolarization of a given preparation was fairly constant between 60 and 180 min in the K-free Ringer's solution, provided each application of Ringer's solution for approximately 3 min was repeated at an interval of 15 min.

The membrane hyperpolarization of sympathetic ganglion cells, which was produced by 5-HT, could be observed only in nicotinized preparations[1]. Hence, the effects of 5-HT on the K^+-activated hyperpolarization were studied in the presence of nicotine (0.12 m*M*) which was present in the superfusing fluid throughout the experiment. When 5-HT ($1–10^{-2}$ m*M*) was added to K^+-free Ringer's solution, the ganglion cells were hyperpolarized (Figure A-2); the 5-HT-induced hyperpolarization was always smaller in K^+-free Ringer's solution than in Ringer's solution[1]. The 5-HT-induced hyperpolarization reached its maximum value within approximately 3 min and thereafter gradually diminished when 5-HT was present for more than 10 min.

The amplitude of the K^+-activated hyperpolarization observed in the presence of 5-HT ($1–10^{-2}$ m*M*) was much higher than that observed in the absence of 5-HT; it was consistently increased (6 experiments) by more than 100% in the presence of 0.1 m*M* 5-HT (Figure A-3). Such an effect of 5-HT was observed as long as 5-HT remained in the solutions and was completely reversible upon its withdrawal.

Ganglion cells were not hyperpolarized but depolarized by 5-HT in the absence of nicotine[1]. In the present experiment, such a depolarization by 5-HT was also observed in the K-free Ringer's solution containing no nicotine. The K^+-activated hyperpolarization was consistently augmented by 5-HT in 4 experiments using 0.1 m*M* 5-HT to the same extent as observed in the presence of nicotine.

No augmentation of the K^+-activated hyperpolarization was observed when creatinine sulfate was used instead of 5-HT creatinine sulfate.

Experiments were also carried out with nerve fibres. In these experiments, desheathed splanchnic nerve trunks were superfused with K^+-free Ringer's solution, and the K^+-activated hyperpolarization was recorded by changing the K^+-free solution to Ringer's solution (Figure B-1). As in the case of the ganglia, the amplitude of the K^+-activated hyperpolarization (1-2 mV) was fairly constant between 60 and 180 min in the K^+-free solution.

Axons of the splanchnic nerve were depolarized by 5-HT ($1–10^{-2}$ m*M*) (Figure B-2) in the presence as well as in the absence of nicotine (0.12 m*M*). No augmentation of the K^+-activated hyperpolarization was observed with 5-HT (Figure B-3) in the presence or absence of nicotine.

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The K^+-activated hyperpolarization of both ganglion cells and splanchnic nerves was reversibly eliminated by the action of ouabain. As seen in Figure A-5 and B-4, the K^+-activated hyperpolarization was completely prevented by ouabain in a concentration as low as 2×10^{-3} mM; a depolarization now occurred when the K^+-free fluid was switched to the Ringer's solution.

The fact that K^+-activated hyperpolarization of both sympathetic ganglion cell bodies and axons was very sensitive to ouabain well supports the concept it was generated by an activation of the electrogenic Na^+-pump[3]. Although the mechanism by which the K^+-activated hyperpolarization of ganglion cells was accelerated by 5-HT could not be clarified, the acceleration did not seem to be due simply to an increase of the membrane resistance; the membrane resistance of ganglion cells measured by intracellular microelectrodes was not increased by 5-HT[5]. Therefore, the present results suggest that 5-HT directly accelerates the electrogenic Na^+-pump in ganglion cells[1]. 5-HT accelerated the K^+-activated hyperpolarization of ganglion cells but not that of nerve fibres. Presumably, the receptors which mediate the effect of 5-HT are located on the membrane of the cell bodies of sympathetic neurons but are absent in their axons.

Zusammenfassung. Die Hyperpolarisation von paravertebralen Ganglien des Ochsenfrosches (*Rana catesbeiana*) in vitro, die durch K^+ nach vorheriger Inkubation in K^+-freier Ringerlösung ausgelöst wird, war in Gegenwart von Serotonin (5-Hydroxytryptamin) deutlich verstärkt. Auf die K^+-bedingte Hyperpolarisation von Axonen des N. splanchnicus blieb Serotonin ohne Einfluss. Es wird angenommen, dass Serotonin die elektrogene Na^+-Pumpe über Rezeptorenstimulation aktiviert, die an der Membran der Zellkörper, nicht jedoch an Axonen sympathischer Neuronen vorhanden ist.

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Electroreceptive Properties of *Silurus glanis* (L.)

After it was well established that sharks and rays[1,2], as well as *Ictalurus nebulosus*[3], possessed a so-called 'passive' (non-electrogenic) electroreceptive system which makes them capable of using electrical cues from their environment, the question arose whether similar receptive properties could be found in other teleosts. In view of the order of magnitude of natural electric fields in freshwater[4], it seems that only a few of the known current density threshold values[5–10], e.g. those of *Clarias*[8], *Lepidosiren*[9] and *Anguilla rostrata*[10,11], are within the physiological range.

In a first attempt to examine further teleost species for the possession of biologically significant passive electroreceptive systems, specimens of the European silurid *Silurus glanis* (L.) were selected (length approx. 30 cm). According to the method described by Peters and Buwalda[3], spikes were recorded from the superficial branch of the lateral-line nerve, which innervates skin receptors of the flanks[12]. According to Herrick[13], this nerve corresponds to the ventral branch of the ramus lateralis X used by Peters and Buwalda[3] in the silurid *Ictalurus nebulosus*.

The results resemble fairly closely those obtained by Peters and Buwalda[3] and consequently show that this superficial branch of the r.lat. X is involved in the reception of weak electrical stimuli. As the adaptation to a DC stimulus current is slow (time constant of several sec), the receptors can be considered tonic. With a homogeneous, vertically applied sinusoidal stimulus current of 3 Hz, the threshold current density was approx. 10^{-10} A/mm^2. The upper limit of the frequency response differed somewhat from that of *Ictalurus*: instead of decreasing rapidly above 10 Hz, the response was quite pronounced up to 25 Hz, at which frequency a certain degree of spike synchronization was observed. With further increasing frequency, the response diminished rapidly.

The above-mentioned results show that *Silurus* possesses an electroreceptive system which may well function in preydetection and/or orientation, as found in *Ictalurus*[14,15].

Zusammenfassung. *Silurus glanis* (L.) hat elektrische Sinnesorgane, deren Eigenschaften mit denen des *Ictalurus nebulosus* übereinstimmen: die Rezeptoren sind tonisch, die obere Frequenzgrenze liegt bei etwa 25 Hz, und der Schwellenwert beträgt ungefähr 10^{-10} A/mm^2. Sie könnten also, wie bei *Ictalurus*, sowohl dem Beutefang als der Orientierung dienen.

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