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MEMBRANE PROPERTIES OF RANVIER NODES FROM SOUTH AMERICAN TOADS AND FROGS *(BUFO MARINUS ICTERICUS* AND *LEPTODACTYLUS OCELLATUS)**

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PROPRIEDADES DA MEMBRANA DOS NÓDULOS DE RANVIER DE RÃS E SAPOS DA AMÉRICA DO SUL (*BUFO MARINUS ICTERICUS* E *LEPTODACTYLUS OCELLATUS*)

RESUMO

Estudaram-se propriedades eletrofisiológicas de membranas excitáveis em alguns anfíbios do Brasil. O presente trabalho refere-se aos resultados obtidos em nódulos de Ranvier de fibras motoras e sensoriais isoladas de *Bufo marinus ictericus* e *Leptodactylus ocellatus.* Empregou-se o método desenvolvido por Nonner (1969).

Esta pesquisa teve por objetivo, comparar a membrana da fibra nervosa desses animais, vivendo em clima quente, com a de *Rana esculenta* e *Xenopus laevis* disponíveis na Europa e também com a de ratos e gatos.

Usaram-se Osciloscópio Tektronix 565 e Camera Robot para os registros.

Os valores absolutos do potencial de membrana foram determinados com fibras cortadas, ao término do experimento, no lado onde se aplicava a corrente.

Incidentalmente, um "duplo nódulo", presente em alguns casos nas fibras de *Bufo,* foi montado na câmara de registro. O internódulo em foco media somente 23 μm de comprimento e 5 μm de diâmetro, semelhante ao citado por Lubinska (1958).

* Published at the occasion of the retirement of Prof. Paulo Sawaya as a modest sign of admiration, friendship and gratitude.

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Número especial em homenagem ao Prof. Dr. Paulo Sawaya, no ano jubilar de seu magistério.

A única diferença distinta entre as fibras sensoriais e motoras é a resposta repetitiva a estímulos de longa duração.

Os resultados obtidos corroboram os de Frankenhaeuser e outros (1959) no que se refere ao comportamento do sistema sódio. O sistema potássio entretanto, apresenta menor retificação que o de *Rana esculenta, Rana ridibunda, Bufo bufo* e *Xenopus laevis.*

Nossos sapos e rãs do Brasil apresentam a relação INa max/IK para VNa de 2.9 em membranas de *Leptodactylus* e 3.9 em membranas da fibra nervosa de *Bufo.*

Figurariam, então, no limite superior dos Anfíbios aproximando-se da faixa dos animais de sangue quente.

Nossos resultados mostram que estes animais são por excelência, apropriados para os experimentos de "Voltage Clamp" em um nódulo de uma única fibra nervosa isolada.

SUMMARY

Electrophysiological properties of excitable membranes were studied in a few brazilian amphibia. The present paper deals with the results obtained on single Ranvier nodes of *Bufo marinus ictericus* and *Leptodactylus ocellatus* motor and sensory fibres with the voltage and current clamp method developed by Nonner (1969).

The purpose of this investigation was to compare the nerve membrane of these animals, living in a warm climate, with the fibres of *Rana esculenta* and *Xenopus laevis* available in Europe and also with the nerve fibres of rats and cats.

A Tektronix 565 oscilloscope and a Robot Camera were used for recording.

The absolute values of the membrane potential were determined with the fibres cut (at the end of an experiment) on the side pool where current was applied.

Incidently, a "double node", in some cases present in *Bufo* fibres, was mounted in our nerve chamber with an intercalated internode of only 23 μm length and 5 μm diameter, resembling the ones presented by Lubinska (1958).

The only distinct difference of sensory fibres compared to motor ones is the repetitive response to long lasting stimuli.

The present results are in good accordance with the results of Frankenhaeuser and others (1959) with respect to the behaviour of the sodium system. The potassium system, however, present less rectification than the one of *Rana esculenta, Rana ridibunda, Bufo bufo* and *Xenopus laevis.* Our Frogs and Toads had a ratio of INa max/IK at VNa of 2.9 for *Leptodactylus* membranes and 3.9 for *Bufo* membranes.

They figure thus at the upper limit of amphibia aproaching the range of warm blooded animals.

Our results show the excellente suitability of these animals for single node voltage clamp measurements.

During a three-months stay at the Instituto de Biociências (São Paulo) and at the Instituto de Biologia Marinha (São Sebastião) we studied electrophysiological properties of excitable membranes in a few brazilian amphibia and marine animals. The present paper deals with the results obtained on single Ranvier nodes of *Bufo marinus ictericus* and *Leptodactylus ocellatus* with the voltage and current clamp method developed by Nonner [1]. The purpose of this investigation was to compare the nerve membrane of these animals, living in a warm climate, with the fibres of *Rana esculenta* and *Xenopus laevis* available in Europe and also with the nerve fibres of rats and cats.

METHOD

The nerve fibres were dissected according to the method described by Stämpfli [2] for *Rana esculenta.* The *Bufo* specimen came directly from the vivarium of the Institute, where they lived in open air and were feeding nightly on the numerous insects attracted by electric light. *Leptodactylus ocellatus* were caught near Santos and kept at room temperature for a few weeks in the animalry of the institute without feeding. They were nevertheless in excellent condition and extremely healthy and strong. Dissection was relatively easy in both species. The toads, however, had a tuffer sheath similar to mammalian nerve. Their fibre diameter was only 12-15 μm just as in big specimen of *Rana esculenta.* The fibrils of the endoneurium are solid and the danger of strangulation of fibres during the dissection is bigger than in European frog and toads. *Leptodactylus ocellatus,* however, is an ideal frog for dissection, presenting fibres of 15-18 μm and little connective tissue.

Bufo fibres in some cases present intercalated internodes as described by one of us in *Bufo bufo* [3] of less than 100 μm length, alternating regularly with long internodes of 2000 to 3000 μm. Incidently, such a "double node" was mounted in our nerve chamber (see Results).

The electronic equipment and the nerve chamber corresponded exactly to the arrangement described by Nonner. The amplifier used was the one calculated for *Xenopus* fibres. During the warm days cooling of the chamber to 20°C was obtained by flowing tap water through the metal block under the chamber and through a heat exchanger where the inflowing solutions could equilibrate with the water temperature before flowing through the stop cock giving access to

the preparation. A Tektronix 565 oscilloscope and a Robot Camera were used for recording. The voltage clamp results were digitalized, corrected for leak and plotted with a Honeywell 516 computer, belonging to the Sonderforschungsbereich — Membranforschung — of the Deutsche Forschungsgemeinschaft in Homburg/Saar. **

The absolute values of the membrane potential were determined by cutting the node under investigation with fine scissors. This procedure was not in all cases successful.

The current clamp records were analyzed from the film records. Action potentials were differentiated electrically to obtain the maximum rates of rise and fall. The durations were taken as time elapsing between maximum rate of rise and fall (cfm. Schmidt and Stämpfli [4]). Current density is calculated by dividing the potential at the output of the feedback amplifier by 10 Ω cm^2. As all values presented in this paper were obtained with the fibres cut on the side pool where current was applied, these values are not easily compared with the values of Frankenhaeuser, who usually did not cut his fibres.

RESULTS

a. *Action potentials*

The action potentials of motor and sensory fibres in both species do not show significant difference of duration, rate of rise, amplitude, absolute threshold potential and resting potential. The values were, therefore, pooled and listed separately for the two species in table 1.

TABLE 1 — Action potentials at 21°C

	amplitude mV	duration ms	maximum rate of rise V/sec	maximum rate of repolarisation V/sec	absolute* threshold level mV	resting potential after cutting mV
Leptodactylus ocellatus	116.8±1.4 (n = 8)	0.73±0.05 (n = 9)	2260±130 (n = 9)	191±18 (n = 6)	19.8±0.9 (n = 7)	60.1±2.9 (n = 4)
Bufo marinus ictericus	120±1.6 (n = 8)	0.75±0.1 (n = 8)	2650±60 (n = 8)	207±16 (n = 8)	19.5±1 (n = 5)	64.1±2.9 (n = 6)

* potential where the rate of rise reached the value 25 V/sec

** The cooperation of Mrs. Doris Nonner is gratefully acknowledged.

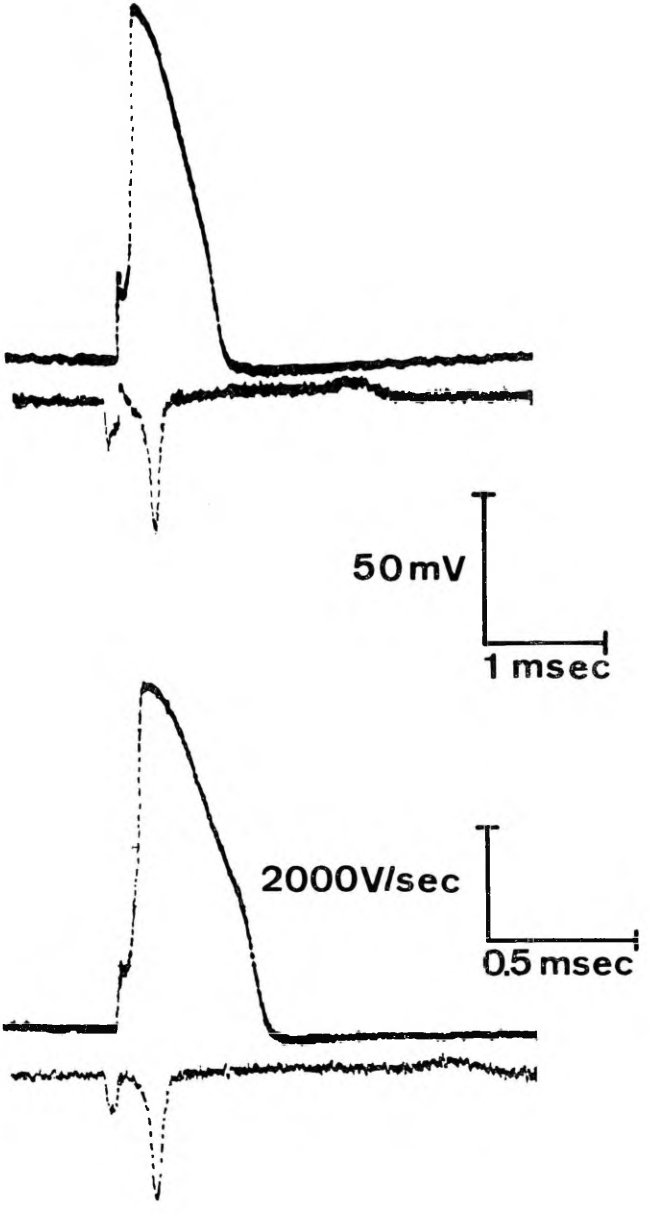

Fig. 1 — Action potentials of motor nerve fibres of *Leptodactylus ocellatus* (upper part) and *Bufo marinus ictericus* (lower part) with corresponding first derivatives. Temperature 21°C.

Typical examples of motor action potentials and of their first derivative are presented in fig. 1. The only distinct difference of sensory fibres compared to motor ones is the repetitive response to long lasting stimuli (fig. 2).

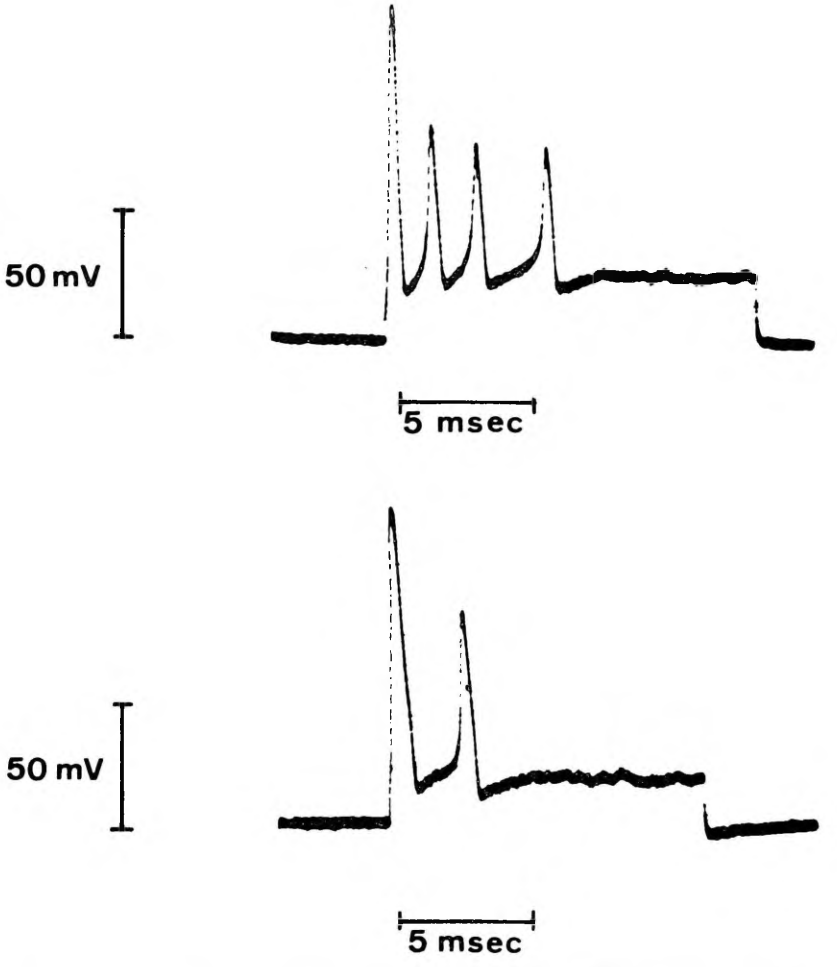

Fig. 2 — Repetitive response of nodes of sensory fibres to long lasting current pulses. Upper part *Leptodactylus ocellatus*, lower part *Bufo marinus ictericus*. Temperature 21°C.

b. *Voltage clamp measurements*

Typical examples of voltage clamp runs and the current voltage curves obtained accordingly are given for *Leptodactylus* in figs. 3 and 4, for *Bufo marinus ictericus* in figs. 5 and 6.

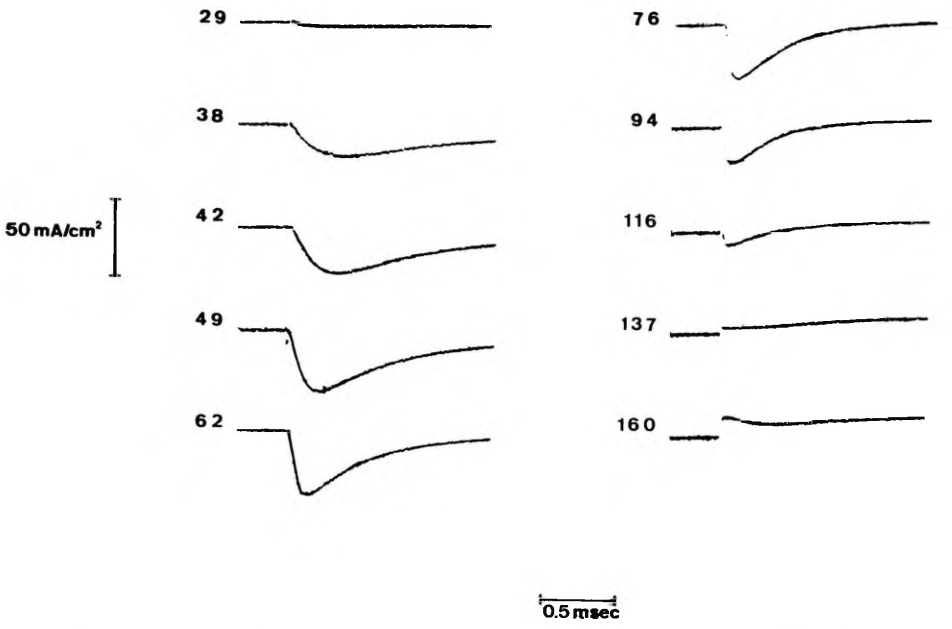

Fig. 3 — Voltage clamp run on a motor fibre of *Leptodactylus ocellatus*. The depolarisation during the voltage pulses is indicated in mV at the beginning of the records. Temperature 21°C.

Both current voltage curves clearly indicate relatively small delayed rectification of the potassium currents compared to the sodium peak inward currents.

c. *Double node*

Fig. 7 represents the voltage clamp currents obtained with one *Bufo* fibre, showing two distinct inward currents, both perfectly graded, clearly indicating two excitable membranes in the nerve chamber compartment. A through microscopical examination of the nerve fibre at the end of the experiment gave proof of the presence of an intercalated internode of only 23 μm length and 5 μm diameter in the nodal compartment resembling the ones presented by Lubinska [5]. * The internodes are in fact so short that sometimes, as in this case, one does not realize the presence of double nodes with an ordinary dissection microscope.

* The help of Prof. Lavallard in taking photographs allowing the measurements of dimensions mentioned in the text is gratefully acknowledged.

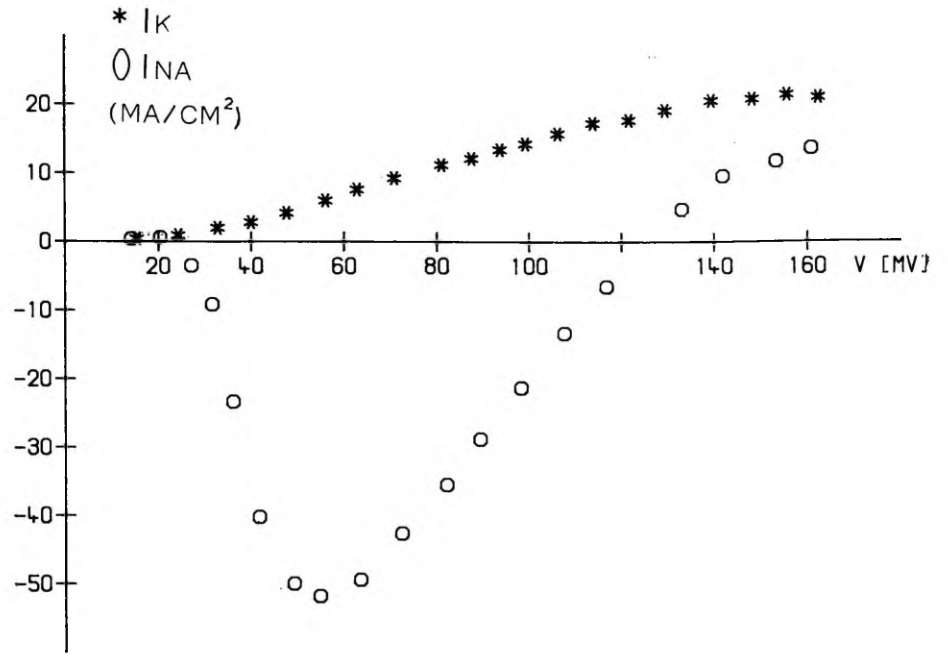

Fig. 4 — Peak sodium currents from fig. 3 and steady state potassium currents (measured near the end of the 50 msec pulses of the same run) plotted against membrane potential. Sodium equilibrium potential about 124 mV Temperature 21°C.

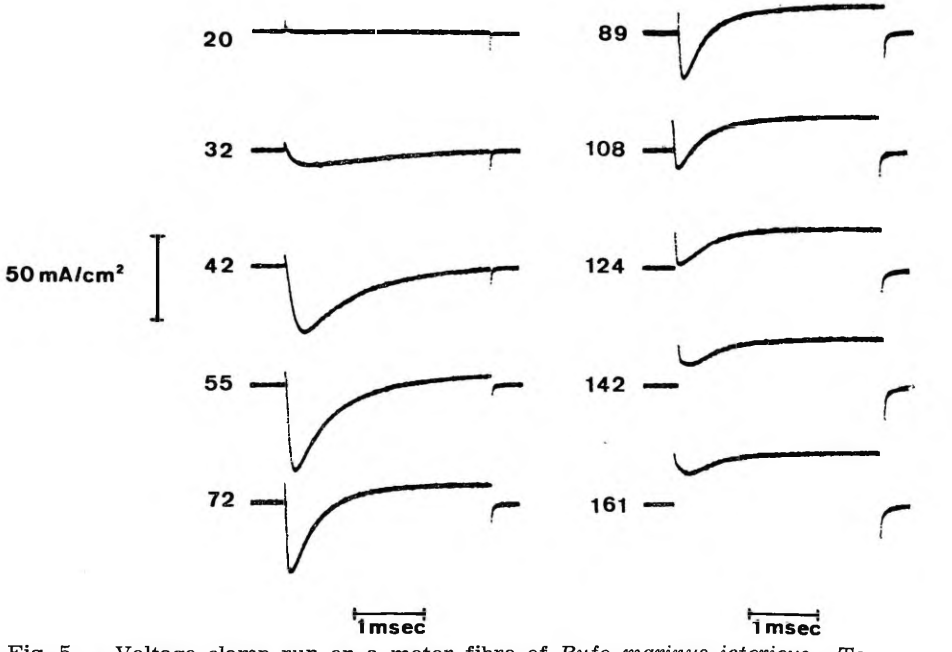

Fig. 5 — Voltage clamp run on a motor fibre of *Bufo marinus ictericus*. Temperature 21°C. (Compare fig. 3).

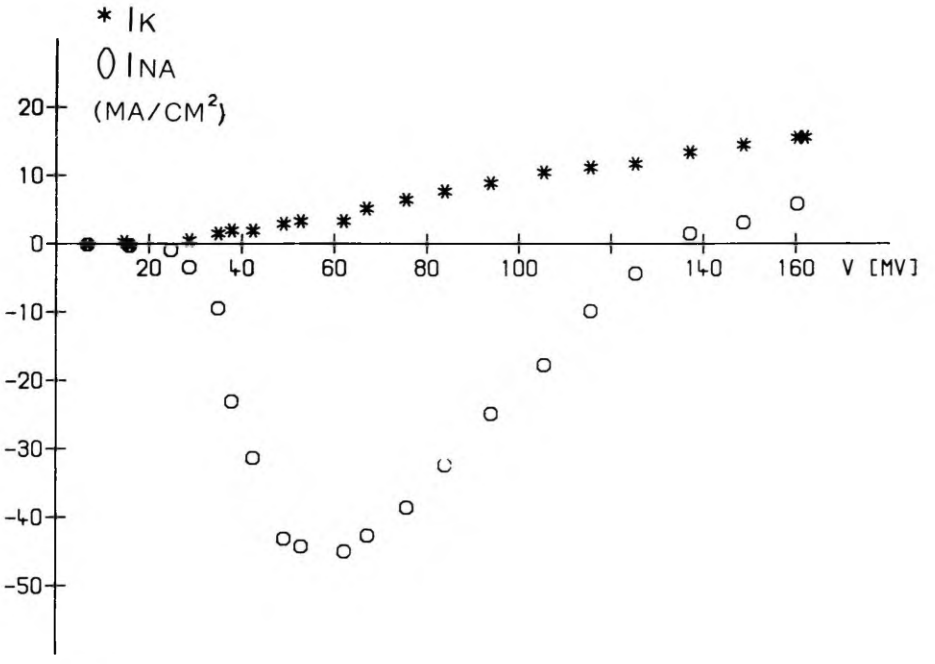

Fig. 6 — Peak sodium currents from fig. 5 and steady state potassium currents (measured during another run on the same fibre near the end of 50 msec pulses). Sodium equilibrium potential about 134 mV. Temperature 21°C.

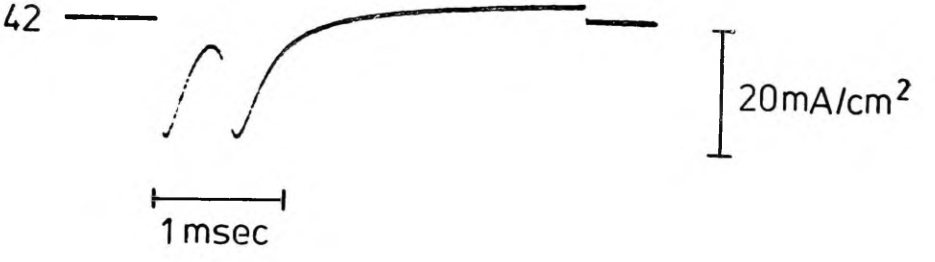

Fig. 7 — Membrane current associated with a depolarizing pulse of 42 mV in a 'double node'. Fibre from *Bufo marinus ictericus*. Temperature 21°C. Note the two peaks in the early inward current indicating a non-synchronous behaviour of both nodes. This is explained by the longitudinal potential drop in the axoplasm between both nodes which is caused by the membrane current of the first node and decreases the pulse applied to the second node as long as the membrane current of the first node is inward. Thus, both components of the inward current are smoothly graded with depolarization

DISCUSSION

The present results are in good accordance with the results of Frankenhaeuser and others [6, 7] with respect to the behaviour of the sodium system. The potassium system, however, presents less rectification than the one of *Rana esculenta, Rana ridibunda, Bufo bufo* and *Xenopus laevis*.

The potassium system seems to be less developed with respect to the sodium system, the more the animals have to live in warm climates or the higher their blood temperature. We have tried to compare the maximum sodium currents obtained in voltage clamp at $h_\infty = 1$ (at potentials between 50 an 60 mV depolarisations from V = 0) with the potassium current obtained at V_{Na} (120 to 130 mV depolarisation from V = 0) found in the literature. Squid and lobster membranes have a ratio of $I_{Na\ max}/I_K$ at V_{Na} of about 1, *Rana esculenta* about 1.4 *, *Xenopus laevis* 2.7 [8], *Rana pipiens* about the same, pigeon 4 [9], rat 5-10. * Our frogs and toads from Brazil had a ratio of 2.9 for *Leptodactylus ocellatus* and 3.9 for *Bufo marinus ictericus*. They figure thus at the upper limit of amphibia, approaching the range of warm blooded animals.

We are aware of the difficulties to use such a ratio as criterion to compare different species. This ratio, however, has the advantage of avoiding the discussion of comparing P_{Na} and g_{Na} and gives a reasonable figure even in cases where exact values of permeabilities or conductances have not been determined.

The considerable difference between the shape of the action potential of sensory and motor fibres, observed in *Rana esculenta* is almost absent in *Bufo marinus ictericus* and in *Leptodactylus* fibres. The main difference between sensory and motor fibres in these animals is the accommodation to long lasting stimuli, which is significantly smaller in sensory fibres and leads to repetitive behaviour. The rates of rise of the action potentials of *Bufo marinus ictericus* fibres and *Leptodactylus* are more than 2000 V/sec at room temperature. They are higher than the ones of *Rana esculenta* and compare well with the ones of the rat [10]. We believe that the differences in amplitude, resting potential and rate of rise in *Bufo* fibres compared to *Leptodactylus* may have their origin in different conditions of the animals, the latter having been kept without food for 2-3 weeks; even then the resting potential values seem relatively low compared to the 71 mV

* own unpublished data

given by Huxley and Stämpfli [11] for *Rana esculenta*. Our method for determining the resting potential is rather crude. The fibre may be pulled and the seal resistances may, therefore, have changed. Furthermore, cutting is only performed at the end of an experiment — often after several runs for current voltage curves or h_∞ curves. The duration of an experiment was at least 30 min — more frequently an hour or more. The low values may thus be explained by the run-down of membrane potential and to its enhancement by excessive ionic currents during the voltage clamp series.

To conclude our results show the excellent suitability of the South American frog *Leptodactylus ocellatus* and of *Bufo marinus ictericus* for single node voltage clamp measurements. Their membrane is in all respects very similar to the one of *Xenopus* and *Rana* except for their low K-rectification which resembles the behaviour of nodal membranes of the pigeon and of the rat.

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