CD_{50} after SKF 525A, from 1.74 at 3 weeks to 3.30 at 6 months. Unless one assumes a change with age in the accessibility or the susceptibility of hepatic enzymes to SKF 525A without any evidence suggesting these alternatives, the best interpretation of the ratio data would again be that of an age-dependent increase in hepatic capacity for strychnine inactivation. These data again point to the interpretation of i.p. strychnine CD_{50} changes with age as results of postnatal ontogenesis of hepatic enzyme activity, as was suggested also by the data of KATO et al.[5,6].

By its chemical nature the volatile convulsant flurothyl is insensitive to metabolic inactivation. The maximal (tonic-clonic) flurothyl response, which corresponds most nearly to the strychnine seizure, was not significantly facilitated by SKF 525A pretreatment. Furthermore, the intensity of response to flurothyl was decreased rather than increased by pretreatment. Therefore, it is not possible to attribute SKF 525A effects on strychnine CD_{50}'s to a direct CNS action.

The relatively slight increase in CD_{50}'s with age in our groups receiving i.v. strychnine and i.p. strychnine plus SKF 525A does not allow the total exclusion of maturational changes in the CNS such as that of the blood-brain barrier postulated by PYLKKO and WOODBURY[1]. However, the data indicate that such events, if occurring after 3 weeks of age, have a much smaller effect on sensitivity to strychnine than was suggested by the results following i.p. injection in the former study.

Résumé. Chez le rat ce n'est pas la perméabilité du système nerveux central, mais le métabolisme du foie qui joue le rôle principal dans la susceptibilité á la strychnine inoculée par voie intrapéritonéale.

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Na-Activated Calcium Efflux in Rabbit Vagus Nerve Fibres

A system which exchanges intracellular calcium against extracellular sodium has recently been described in mammalian heart[1] and squid giant axons[2]. It seems that extrusion of calcium is driven by influx of sodium along a concentration gradient; a common carrier has been postulated[1] for the outward movement of calcium and part of sodium entry. Evidence from the present paper suggests the existence of a similar system in mammalian nerve fibres. The experiments were performed on rabbit vagus nerves, which consist mainly of non-myelinated fibres[3] and thus are particularly suitable for ion-exchange-studies.

Desheathed vagus nerves were incubated for 2 h at 37 °C in Locke's solution containing tracer amounts of radioactive calcium. After rinsing for 20–30 sec in tracer-free medium, the ends of the nerves were covered with vaseline, fixed in a small tube (volume 0.35 ml) and the preparations washed by a constant flow (1 ml/min) of bathing solution. The composition of this solution could be changed rapidly by switching to another reservoir. Washing fluid fractions of 3 ml were collected in counting vials and, after addition of 2 drops of 1 *M* oxalate, dried at 90 °C. At the end of the experiment, the nerves were homogenized in 6 ml Locke's, transferred to a counting vial and dried. 3 ml of scintillator fluid (250 mg POPOP, 2 g PPO, 1000 ml toluene) was added to the dry samples and radioactivity was determined in a Packard Tricarb scintillation counter. Different amounts of ^{45}Ca comparable to those released by the nerve during a 3 min period proved to be perfectly measurable under these conditions. Since self-absorption varied with certain solutions, it was necessary to establish correction factors for quenching. Alternatively, in the experiments related to the effect of temperature on Ca-efflux, the nerves were successively immersed in a series of vials containing 3 ml Locke's. Radiocalcium was then measured as above. From the amounts of ^{45}Ca found in the samples, the rate-constant for calcium loss was calculated and plotted on semilogarithmic paper. Locke's solution was of the following composition (m*M*): NaCl 153, KCl 5.6, $MgCl_2$ 0.5, $CaCl_2$ 2.2, *tris* 1, glucose 5.5. In several experiments Na^+ of the solution was replaced by equimolar amounts of Li^+ or choline.

The curve represented in Figure 1 results from a standard washout experiment at 21 °C and shows the rate-constant of Ca-efflux as a function of time. A similar result has been obtained by KEYNES and RITCHIE[3], who applied drugs and electrical stimulation to rabbit vagus nerves during the second hour of calcium washout. In the present experiments, however, solutions which were to be examined for their effect on calcium loss were applied from min 24 to min 60 after beginning of the experiment. The curve is far from being exponential in this region, but the interval was chosen on the assumption that this part of outflux comes preferably from the easily accessible C-fibres.

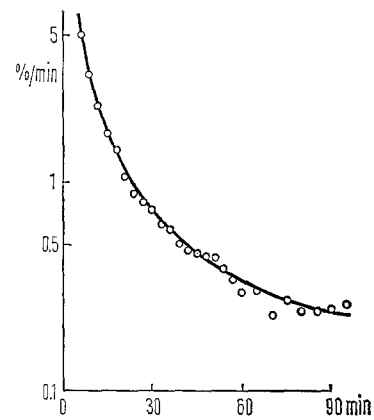

Fig. 1. Efflux of Ca from desheathed rabbit vagus nerve as a function of time. Temperature 21 °C.

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Figure 2 shows the effect of removing sodium from the washing solution, this ion being replaced by choline. The rate-constant fell to about 60% of its previous value, it promptly increased upon readmittance of sodium containing solution. These results indicate a partial coupling between the outward movement of calcium and sodium entry; they are similar to those found in mammalian heart and squid giant axon. There was, however, only a slight initial drop when sodium was replaced by lithium; upon reintroduction of external sodium, the Ca-outflux failed to rise. This may be in relation to the fact that Li is heavily accumulating in rabbit vagus during incubation in Li-Locke[4] and thus could prevent sodium influx to occur at a normal rate.

The results from another series of experiments suggest that the system that exchanges Na for Ca is different from the Na-carrying system[5] mediating the Na-influx during the action-potential. Thus a number of drugs known to influence the Na-carrying system did not alter Ca-efflux (see Table). Further, the Ca-Na exchange was not influenced by caffeine, a substance which increases the Ca-efflux in frog muscle[6]. This seems reasonable since caffeine apparently acts on the intracellular vesicles rather than on the cell membrane.

ROJAS and HIDALGO[7] saw a marked increase of calcium outflow from Dosidicus gigas giant axons when applying metabolic inhibitors. HODGKIN and BLAUSTEIN[2] found an increase in efflux from giant axons caused by cyanide at a time when intracellular ATP was low and attributed this effect to mobilization of calcium from intracellular stores. In rabbit vagus nerves application of CN^- produced no increase in Ca-efflux, 2,4-dinitrophenol seemed to decrease the efflux of Ca. An explanation for this may be the abolition of the transmembrane sodium gradient by metabolic inhibitors: A rapid accumulation of sodium has been shown to occur in rabbit vagus nerves under the action of these substances[8].

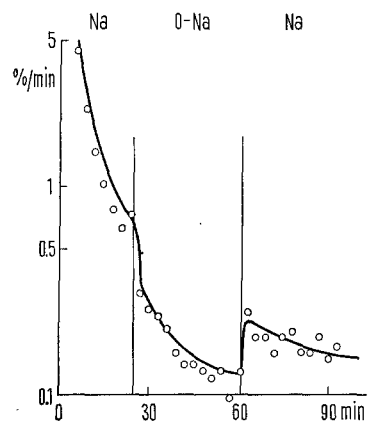

Fig. 2. Effect of replacing extracellular Na by choline on Ca-efflux from desheathed rabbit vagus. Temperature 22 °C.

Substances applied to ^{45}Ca loaded rabbit vagus nerves

Substance	Concentration	pH	Effect on Ca-efflux	
Veratridine	10^{-5} g/ml	7.2	–	(2)
Veratridine	10^{-4} g/ml	7.7	–	(1)
Tetrodotoxine	10^{-6} g/ml	7.1/7.9/7.5	–	(3)
Tetracaine	1 m*M*	7.4/6.6	–	(2)
Caffeine	5 m*M*	7.1	–	(1)
2,4-Dinitrophenol	0.4 m*M*	6.5	slight decrease	(1)
Cyanide	2 m*M*	6.5	–	(2)

(Number of experiments in brackets).

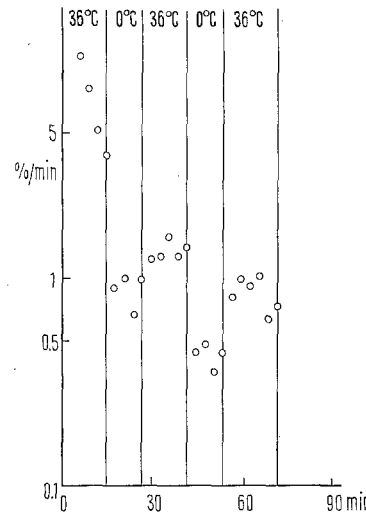

Fig. 3. Effect of temperature on Ca-efflux from desheathed rabbit vagus.

Experiments on giant axons[2,7] indicate a high Q_{10} for calcium extrusion from this tissue. To evaluate the effect of temperature on Ca-efflux from rabbit vagus, bathing solutions of 36 and 0 °C were used alternatively in the experiment shown in Figure 3. After correction for the decrease of outflux occurring at constant temperature, a Q_{10} of 1.4 was calculated; 2 other experiments gave similar low values. This finding is in contrast to the observations on giant axons[2,7] and to those in human red cells[9]. The corresponding amount of activation energy (5500 cal/mole) agrees, however, closely with the results on mammalian heart where 5900 cal/mole are necessary for Ca-extrusion. The low values suggest that calcium outflow from these tissues is not driven by metabolic processes[10].

Zusammenfassung. Die vorliegenden Resultate deuten darauf hin, dass aus marklosen Nervenfasern ein passiver Austausch von intrazellulärem Kalzium gegen extrazelluläres Natrium erfolgt, und zwar mittels eines Überträgersystems, das von dem für die Erregung notwendigen Na-übertragenden System verschieden ist.

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