to 1.4 msec ($N = 42$, mean 0.95 msec) and the duration varied from 5 msec to 30 msec (mean 10 msec). In some NRTP neurons, EPSPs were induced following IP as well as DBC stimulation. Collision experiments illustrate (Figure E–H) that IP induced EPSPs were blocked by conditioning DBC stimulation at stimulus intervals of less than 0.5 msec. A partial recovery was observed at 0.7 msec and full recovery at 1.2 msec inter-stimulus intervals. Considering the latencies of DBC and IP induced EPSPs and the results of collision experiments, NRTP cells are monosynaptically activated by axons of IP cells via the brachium conjunctivum. This conclusion is in agreement with anatomical observations[1–4]. Conduction velocity of the IP axons impinging on the NRTP cells was estimated by measuring differences in latencies between IP and DBC induced EPSPs and the distance between stimulation sites. The conduction velocity thus calculated from 6 intracellularly recorded NRTP cells ranged from 25M/sec to 50 M/sec (mean 33 M/sec). At times, the stimulation of IP resulted in antidromic firing of the NRTP neurons followed by monosynaptic EPSPs or synaptically activated spikes. This could be interpreted as supportive evidence for a reciprocal pathway between the IP and pontine nucleus as suggested by TSUKAHARA and BANDO[9].

Some of the IP induced EPSPs were reversed by application of depolarizing currents through the recording microelectrode (Figure I–L). Figure I is a control record of an IP induced EPSP. By successive increases in depolarizing current the amplitude of the EPSPs decreased (Figure J). The transmembrane potential was almost at its reversal potential at 5.1 nA (Figure K) and was reversed at 7.5 nA intensity (Figure L). These results would indicate that some excitatory synaptic terminals of IP axons are located on or near the soma of the NRTP cells[10].

Figure M shows an example of monosynaptically induced EPSPs following CP stimulation. The latency of CP induced EPSPs varied from 0.7 msec to 1.5 msec ($N = 10$, mean 1.1 msec) and the duration from 7–26 msec (mean 13 msec). This average latency of 1.1 msec is similar to that of pontine nuclear cells activated by CP stimulation[9]. Direct cortical inputs to the NRTP cells have been observed by anatomical methods[11–14].

In conclusion, the present data show, in agreement with previous anatomical observations, that the NRTP neurons receive direct excitatory inputs from the cerebellar nucleus via the brachium conjunctivum and from the cerebral peduncle. Some electrophysiological characteristics of the NRTP cells are different from those of the LRN cells[15,16] indicating functional differences between these precerebellar nuclei in terms of cerebellar operation.

Résumé. On a enregistré les potentiels electriques mono- et poly-synaptiques (EPSPs) des cellules du nucleus reticularis tegmenti pontis en stimulant le nucleus interpositus (NI), le brachium conjunctivum (BC) et le pédoncule cérébelleux. Les expériences de collision ont montré que des axones des cellules NRTP activent leurs neurones via BC. Quelques potentiels postsynaptiques exitateurs induits par le nucleus interpositus ont été inversés par un courant dépolarisant appliqué au voisinage de la microélectrode de dérivation, ce qui indique que les terminaisons synaptiques d'axones NI se trouvent près du sommet des cellules NRTP.

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[10] W. RALL, J. Neurophysiol. *30*, 1138 (1967).
[11] A. BRODAL and P. BRODAL, Expl. Brain Res. *13*, 90 (1971).
[12] H. G. J. M. KUYPERS, J. comp. Neurol. *110*, 221 (1958).
[13] H. G. J. M. KUYPERS and D. G. LAWRENCE, Brain Res. *4*, 151 (1967).
[14] G. F. ROSSIE and A. BRODAL, J. Anat. *90*, 42 (1956).
[15] S. T. KITAI, H. HATADA and W. CASEY, Brain Res. *43*, 629 (1972).
[16] S. T. KITAI, D. T. KENNEDY, J. F. DEFRANCE and K. HATADA, Expl. Brain Res., accepted for publication (1974).
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Influence of Altered Sterol Composition on (Na^+, K^+) ATPase Activity of Cardiac Sarcolemma

It was shown that in experimental myopathy induced by 20.25-diazacholesterol the activity of (Na^+, K^+) ATPase in membranes of different tissues is increased[1–3]. It was supposed that this increase is due to the incorporation of desmosterol in the different membranes; 20.25-diazacholesterol inhibits desmosterol reductase and therefore desmosterol is accumulated in the treated animals[4].

In this study we tried to find a correlation between the replacement of cholesterol by desmosterol and the increase of specific (Na^+, K^+) ATPase activity in cardiac sarcolemma. 4 groups of male Wistar-rats were studied over a period of 30 days. Group A served as control and received 0.2 ml water by an oesophageal cannula, group B was treated with 10 mg 20.25-diazacholesterol dihydrochloride in 0.2 ml water, group C corresponds to group B but additionally had 2% cholesterol in the standard diet, group D was treated with a suspension of 20 mg triparanol in 0.2 ml water – this substance is also known to inhibit cholesterol biosynthesis at the step of interconversion from desmosterol to cholesterol[5]. All groups of rats were treated daily with the above-mentioned substances. After intervals indicated in the Table, the animals were sacrificed by aortic puncture, cardiac sarcolemma was then prepared according to DIETZE and HEPP[6] and sterol analysis was performed as described earlier[1] in a varian 1400 gaschromatograph equipped with a 3% OV-17 column.

[1] J. B. PETER and W. FIEHN, Science *179*, 910 (1973).
[2] W. FIEHN, E. KUHN and I. GELDMACHER, FEBS-Lett. *34*, 163 (1973).
[3] W. FIEHN, D. SEILER, E. KUHN and D. BARTELS, submitted for publication.
[4] R. E. COUNSELL, P. D. KLIMSTRA and R. E. RANNEY, J. med. Pharmac. *5*, 124 (1962).
[5] T. R. BLOHM and R. D. MACKENZIE, Arch. Biochem. Biophys. *85*, 245 (1959).
[6] G. DIETZE and K. D. HEPP, Biochem. biophys. Res. Comm. *44*, 1041 (1971).

ATPase activities (μmoles P liberated/mg protein × h) in cardiac sarcolemma and percent desmosterol in total sterols of heart muscle of rats

Days of treatment		5	9	12	16	19	23	26	30
Group A	Mg^{2+}ATPase	5.11	6.19	6.90	6.19	5.00	6.37	4.80	6.85
	(Na^+, K^+) ATPase	1.85	2.80	2.77	3.03	2.12	3.27	2.25	3.60
	Stimulation (%)	36.2	45.2	40.0	49.0	42.7	51.3	46.9	52.6
	Desmosterol (%)	0	0	0	0	0	0	0	0
Group B	Mg^{2+}ATPase	6.30	5.80	6.41	5.24	5.24	5.24	4.10	7.00
	(Na^+, K^+)ATPase	3.00	3.80	4.60	5.42	4.80	5.36	4.07	7.65
	Stimulation (%)	47.5	65.5	71.6	103.4	91.7	102.3	99.0	109.3
	Desmosterol (%)	23	38	47	74	68	77	80	81
Group C	Mg^{2+}ATPase	5.60	6.43	6.48	5.96	5.66	6.66	6.02	7.00
	(Na^+, K^+)ATPase	2.80	3.20	2.55	3.13	2.46	2.97	3.02	3.25
	Stimulation (%)	50.0	50.0	39.4	52.5	43.4	44.5	50.1	46.5
	Desmosterol (%)	8	13	20	24	10	15	16	18
Group D	Mg^{2+}ATPase	5.75	5.42	6.30	5.77	5.60	—	—	6.76
	(Na^+, K^+)ATPase	2.99	3.52	3.78	4.40	4.30	—	—	5.43
	Stimulation (%)	52.0	65.0	60.0	76.3	76.8	—	—	80.1
	Desmosterol (%)	14	29	35	46	30	—	—	54

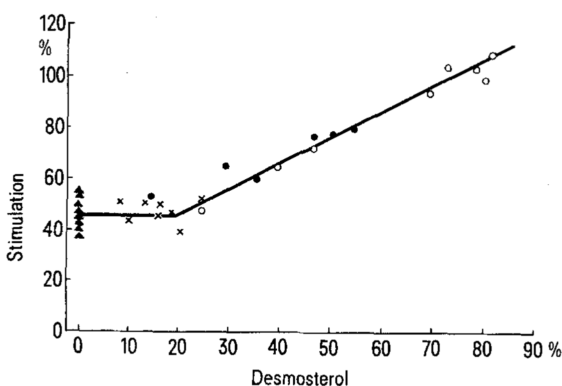
A) controls; B) 20.25-diazacholesterol treated; C) = B) plus 2% cholesterol in the standard diet; D) triparanol treated.

(Na^+, K^+) ATPase was assayed in a medium containing: 60 mM NaCl, 6 mM KCl, 0.5 mM EGTA, 3 mM $MgCl_2$, 3 mM *Tris*-ATP, 30 mM *Tris*-HCl pH 7.0, sarcolemmal protein, 0.2 mg/ml; total volume 2 ml; 37 °C. Incubation time 30 min. To determine the basal, Mg^{2+} ATPase, NaCl and KCl were replaced by 66 mM cholinchloride. Phosphate liberated from ATP was determined according to FISKE and SUBBA ROW[7].

As can be seen from the Table, there are no significant differences with regard to the basic, Mg^{2+}-dependent ATP ase activity. Addition of Na^+ and K^+ results in a stimulation, this alkali stimulated part being completely inhibited by 5×10^{-4} M ouabain.

As described earlier[3], the specific (Na^+, K^+) ATPase was stimulated by treatment with 20.25-diazacholesterol. The effect of 20.25-diazacholesterol was abolished by addition of 2% cholesterol to the diet. Treatment with triparanol also stimulated the specific (Na^+, K^+) ATPase.

It can be seen that the observed increase in group B and D is clearly dependent on the time of treatment, whereas in group A and C specific activity remained low. It also is shown in the Table that this increase of (Na^+, K^+) ATPase activity is accompanied by increasing desmosterol accumulation.



Dependency of (Na^+, K^+) ATPase stimulation of cardiac sarcolemma on the percentage of desmosterol in the sterol fraction of the heart of rats. △, controls (group A); ○, 20.25-diazacholesterol treated (group B); ×, 20.25-diazacholesterol treated plus 2% cholesterol in standard diet (group C); ●, triparanol treated.

The correlation between the percentage of stimulation and the percentage of desmosterol in total sterols of the heart is shown in the Figure. While changes in desmosterol percentage up to 15–20% do not affect (Na^+, K^+) ATPase activity significantly, there is a linear correlation at higher desmosterol percentages in the 20.25-diazacholesterol and the triparanol treated rats as well.

Since total sterol content was only minimally influenced by the treatment, changes in (Na^+, K^+) ATPase activity demonstrated here should be causally related to the replacement of cholesterol by desmosterol in heart plasma membrane. From the results obtained for group C, where virtually little desmosterol was found and no increase in enzyme activity could be observed, one can conclude that 20.25-diazacholesterol itself has no effect on membranal (Na^+, K^+) ATPase activity.

Zusammenfassung. Behandlung mit 20,25-Diazacholesterin bzw. Triparanol führt bei Ratten zu einer Anhäufung von Desmosterin im Herzmuskel. Gleichzeitig kommt es zu einer Erhöhung der spezifischen (Na^+, K^+)-ATPase Aktivität des Herzmuskelsakolemm. Der Zusammenhang dieser beiden Phänomene wird diskutiert.

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[7] C. H. FISKE and Y. SUBBA ROW, J. biol. Chem. *66*, 375 (1925).

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