Boiling of nephrectomized dog plasma at pH 4.9 for 10 min prior to dialysis decreased the binding capacity by 83% (Figure 3). There was no change in the binding capacity of nephrectomized rat plasma following dialysis at 5° to 50°C.

Seven commercially available Cohn fractions of normal human plasma showed some capacity to bind angiotensin (Figure 4). Since each fraction was tested at a concentration equal to the total protein concentration of the dialyzed plasma of nephrectomized dogs (40 mg per ml), the binding capacity of these fractions, excepting fraction IV-4 (α-β-globulin) and fraction V (albumin), can be considered insignificant when compared to the binding capacity of whole plasma. These 7 Cohn fractions were also studied in a concentration of 28 mg/ml and incubated with 2.5 pg angiotensin I labeled with I^{125} for 2 h, at 37°C, in the presence of *tris* buffer with lysozyme (100 mg/100 ml. Free angiotensin was then separated from the bound by charcoal absorption. Three of the fractions showed capacity to bind angiotensin: human fraction IV-1 (α-globulin) bound 46% of the radioactive material, human fraction III (β-globulin) bound 30% and, human fraction III-0 (β-lipoprotein) bound 28%. Similar results were obtained when radioactive angiotensin II was used. The difference in the results obtained by these 2 methods is confusing, but may be due to the difference in the molecular conformation between angiotensin labeled with I^{125} and unlabeled angiotensin. Furthermore, we were unable to displace the radioactive angiotensin I or II bound to the α-globulin proteins by adding unlabeled angiotensin, even using amounts as high as 1 ng.

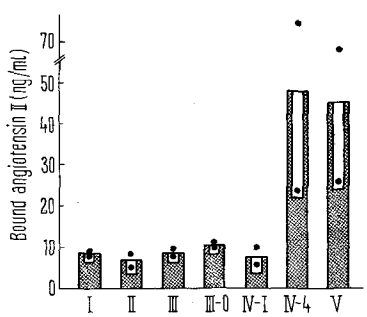

Fig. 4. Angiotensin II binding capacity of Cohn's fractions of human plasma. The protein composition of each fraction is: I, fibrinogen; II, γ-globulin; III, β-globulin; III-0, β-lipoprotein; IV-1, α-globulin; IV-4, α-and β-globulin; V, albumin.

The amount of angiotensin used in the equilibrium dialysis study was far above the physiological level. Concentrations of 20 to 100 pg of angiotensin per ml of plasma have been reported for normal human plasma[7-9]. The amount of binding of angiotensin which we are observing, 30 to 50 ng/ml, is incredibly great when compared to these normal circulating levels. Although binding of radioactive angiotensin by the human α-globulin fraction was observable in an ionic environment, the fact that the bound angiotensin could not be displaced indicates that the binding is not specific. These results, together with the observation that with equilibrium dialysis no measurable binding by the assay used was detectable in the presence of saline or buffer, precludes the postulation of any definite physiological significance for this binding. However, since dialysis against distilled water is commonly used for the elimination of angiotensin from plasma, our observations may have some technical significance. The non-dialyzability of the pressor substance found in renal vein blood plasma by HELMER et al.[1] and GROLLMAN[2] and which seems to be pharmacologically similar to angiotensin, may be due to this binding phenomenon. This possibility has been suggested by Schweikert et al.[10]. Since boiling at pH 4.9 destroys most of the binding activity of plasma, and pH has an influence on the degree of binding, leads us to think that plasma proteins are the factors that bind angiotensin.

Résumé. La liaison de l'angiotensine par les protéines plasmatiques est démontrée par dialyse équilibrée. L'angiotensine radioactive liée ne put être déplacée par l'angiotensine non radioactive, ce qui diminue la signification physiologique de cette liaison.

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Spontaneous Transmitter Release from Nerve Endings and Contractile Properties in the Soleus and Diaphragm Muscles of Senile Rats

Senile atrophy proceeds at a different rate in different muscles[1,2]. This might be due to secondary changes in the skeletal muscle related to associated disturbances, especially joint diseases, differences in androgen sensitivity as indicated by the marked atrophy of the levator ani muscle after castration[3], or to differences in degree of motor activity. In the senile levator ani and extensor digitorum muscles of the rat, both fast muscles, a considerable reduction of spontaneous transmitter release and prolongation of contraction time[4,5], apparently related to decrease of myosin ATPase activity[6], has been observed. Marked reduction of motor activity is also very conspicuous in man, as demonstrated by decreased frequency discharge in motor units[7] and the obvious decrease in force of muscle contraction in the elderly age group[8].

The diaphragm muscle is forced to continuous regular neuromuscular function connected with the respiratory act, and it appeared of interest to compare the changes in spontaneous transmitter release and contractile behaviour in the diaphragm and the soleus muscle of old rats.

Materials and methods. For recordings of the frequency of miniature endplate potentials (MEPP), muscles of young and old rats were dissected and placed in a flat chamber, submersed in LILEY's solution[9]. 3–4 animals were used in each series and MEPP from 32 and 51 muscle cells from the diaphragm and 30 and 51 muscle cells from

Frequency of transmitter release/sec and contraction properties in msec in soleus and diaphragm muscles of young (3 months) and old (30–33 months) animals

		Frequency of transmitter release/sec	%	FCT	%	HCT	%	TCC	%	HRT	%	LP	%
Soleus	Y	1.52±0.10	−92.1	35.0±1.2	+18.9	12.8±0.43	+17.2	5.2±0.39	+32.2	38.2±0.95	+41.1	3.4±0.16	+23.5
	O	0.12±0.01		41.6±0.7		15.0±0.22		11.5±0.39		53.9±1.0		4.2±0.14	
Diaphragm	Y	3.82±0.16	−54.7	16.1±0.4	+28.6	8.2±0.1	+14.6	5.4±0.17	+18.5	14.2±0.6	+57.0	3.0±0.1	+26.7
	O	1.73±0.08		20.7±0.6		9.4±0.4		6.4±0.2		22.3±1.0		3.8±0.13	

FCT, full contraction time (time to peak); HCT, half relaxation time; TCC, maximal rate of tension development; HRT, half relaxation time and LP, latency period. Absolute and relative (%) values of these parameters are indicated, the values of the muscles of young animals being 100%. Number of observations on contraction properties was 7 for both the diaphragm and soleus muscles.

the soleus muscles respectively were recorded by the usual microelectrode technique[10]. MEPP were photographed off the screen of an oscilloscope, transformed to a regular pulse[11] and their frequency/sec was counted for 180–300 sec from each fibre by a Tesla decadic counter.

Contractile properties of the whole soleus and of strips of the diaphragm muscles from 3-month-old rats, i.e. at a time when contraction times in these muscles are shortest[12], and 30–33-month-old rats were studied in vitro. The muscles were set up in a chamber with Pt electrodes for massive stimulation[13], the temperature of the tissue culture medium being 37°C. The twitch responses starting from the same (optimal) resting tension were monitored on an oscilloscope and recordings of latency period (time from stimulus artefact to first mechanical response), full- (time to peak), half-contraction time, half relaxation time and maximal rate of twitch tension development were made by an automatic analyzer of muscle contraction properties[14]. The maximal rate of tension development is expressed as a time constant, independent of amplitude of contraction referring to speed of tension development in msec in the middle (25–75%) part of the ascending contraction curve where a linear slope can be assumed.

Results and discussion. The Table shows the decrease of frequency of MEPP in the diaphragm and soleus muscle fibres in young and old animals, and the changes in contractile properties in these 2 muscles during old age.

In both cases, there is a prolongation of contraction time, rate of maximal twitch tension development, and half relaxation time in the muscles of the old animals. Prolongation of contraction time in muscles of old animals has been found also in the levator ani and extensor digitorum longus muscle[5,12]. It can be seen that the slowing down of contraction properties in both muscles is very similar in the soleus and diaphragm muscle. However, the differences between these 2 muscles are very conspicuous in respect to decrease of frequency of MEPP, which is considerably more pronounced in the soleus than in the diaphragm muscle.

It can be concluded that the reduction of frequency of spontaneous transmitter release, which apparently occurs in all muscles[5], is relatively much less pronounced in the diaphragm than in the soleus muscle and this may be due to the higher usage of these muscle fibres related to the continuous respiratory activity. The slowing of contraction properties observed so far in all muscles we have examined[5,6,12] appears to be characteristic for the ageing process; however, the differences in the slowing down process of contractile behaviour between diaphragm and soleus muscle are very small, whereas the differences in decrease of frequency of transmitter release are very marked.

The relatively less pronounced reduction in spontaneous transmitter release in the diaphragm muscle during old age provides evidence for the importance of increased activity on the aging process, a relation which in man has remained uncertain[15,16]. The observation that in old age frequency of spontaneous transmitter release is much more affected than contraction properties suggests that these 2 mechanisms are not necessarily directly connected, and/or that frequency of nervous impulse activity is more or primarily operative in regulation and development of speed of muscle contraction than previously suggested[17].

Zusammenfassung. Die Frequenz der spontanen Mediatorfreilegung von Nervenendigungen und die Kontraktionsgeschwindigkeit sinken im Soleus- und Zwerchfellmuskel während des Alterns von Ratten. Die Frequenzerniedrigung ist weit stärker ausgeprägt im Soleus als im Zwerchfellmuskel, während die Unterschiede in den Kontraktionsgeschwindigkeiten unbeträchtlich sind. Die relativ geringe Senkung der ACh-Freisetzung im senilen Zwerchfellmuskel wird zur intensiven Nerv-Impulsaktivität beim Atmungsprozess in Beziehung gebracht.

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