The effect of tetrodotoxin on the rate of hydrolysis of acetylcholine in the isolated vagal heart system

Acetylcholine (g)	Rate of hydrolysis Without TTX (g/sec)	With TTX (g/sec)	% of control
1×10^{-8}	8.3×10^{-10}	2.8×10^{-10}	34
1×10^{-8}	7.9×10^{-10}	3.3×10^{-10}	42
1×10^{-8}	8.1×10^{-10}	4.8×10^{-10}	59
1×10^{-8}	8.3×10^{-10}	4.8×10^{-10}	57
1×10^{-8}	8.3×10^{-10}	3.9×10^{-10}	47
2×10^{-8}	9.4×10^{-10}	6.5×10^{-10}	69
2×10^{-8}	8.9×10^{-10}	6.6×10^{-10}	74
2×10^{-8}	9.2×10^{-10}	4.9×10^{-10}	53
2×10^{-8}	9.3×10^{-10}	5.2×10^{-10}	56

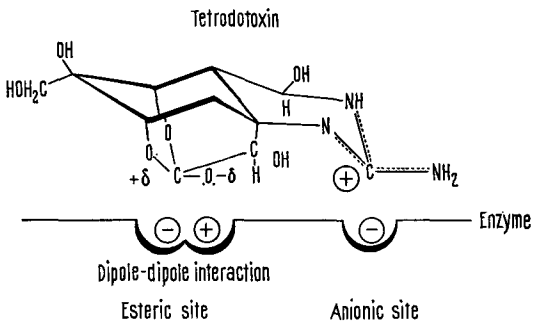

Fig. 4. Model of binding of TTX molecule for active sites of acetylcholinesterase by dipole-dipole interaction at the esteric site and electrostatic forces at the anionic site.

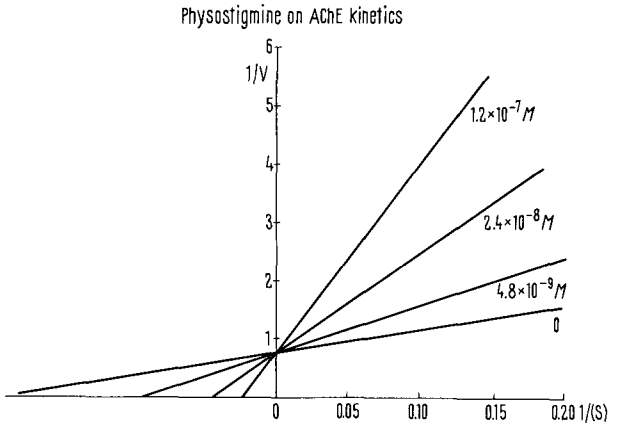

Fig. 3. Double reciprocal plot of 1/v versus 1/(S) at various concentrations of physostigmine.

lines becomes increasingly steeper than the control. That intercepts at the 1/v axis of the control and experimental lines meet at the same spot indicates that TTX is a specific competitive inhibitor for the AChE in situ. For comparison, Figure 3 shows a similar Lineweaver and Burk plot at various concentrations of physostigmine. The striking resemblance between Figure 2 and Figure 3 infers that TTX and physostigmine act on the same enzyme. The apparent K_m for TTX is $2.6\times10^{-6}M$ and that for physostigmine is $4.2\times10^{-6}M$. The K_i for TTX is 1.8×10^{-11} and that for physostigmine is 1.7×10^{-8}. Thus it indicates that TTX binds AChE stronger than physostigmine. Consequently, TTX is a very effective blocking agent for AChE at very low concentration.

A plausible explanation of this remarkable affinity of TTX for AChE may be due to the several characteristic features of TTX molecule. It is generally held that the usual biological active molecules are bound to the active portion of the membrane by 3 forces, namely van der Waals forces, dipole-dipole interactions and electrostatic bindings. With a highly reactive carbonyl group at C_{10} in the TTX molecule (Figure 4), the electron cloud at oxygen atom is sufficiently dense to act as an electron donor capable of forming hydrogen bonds with the esteric sites of AChE molecules of the membrane. The quanidyl group is relatively positive in comparison with the rest of the TTX molecule and fit into the anionic site of the AChE molecule. Since TTX molecule protracts itself as a bird-cage configuration, it is quite conceivable that TTX blocks the 2 active sites more effectively than other AChE inhibitors[12].

Zusammenfassung. Am isolierten Vagus-Herzen von *Rana pipiens* wird die Wirkung der Vagusreizung gemessen: Konzentrationen unter 4×10^{-10} g/ml Tetrodotoxin erhöhen die vagale Reizungswirkung, nicht aber die Herzkontraktionen.

W. Young

Bio-Medical Division, Lawrence Radiation Laboratory, University of California, Livermore (California 94550, USA), 29 December 1969.

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Effect of EDTA on the Distribution Pattern of Acetate-^{14}C in Rats

It has been shown previously[1,2] that following injection of ^{14}C-labelled ethylenediaminetetraacetic and diethylenetriaminepentaacetic acids (EDTA and DTPA) the accumulation of ^{14}C in the liver and kidneys is markedly higher with lower dose of the chelate. This might suggest a dose-dependent deposition of chelating agents and/or of their metabolites and/or of low molecular radioactive contaminants present in the ^{14}C-stock solution[3,4]. Since EDTA and DTPA are broadly used for enhancing excretion of incorporated metals, the present investigation was undertaken in order to elucidate the mechanisms responsible for the effect observed.

Adult female albino rats (weighing 185–200 g, 5 per group) were injected i.v. with 2 μCi of sodium acetate-2-C-14 (20–50 mCi/mmol) alone or with varying amounts of disodium calcium chelate of EDTA. The animals were sacrificed 2 h later and distribution of ^{14}C was determined by methods described previously[2].

As shown in Table I, there are no differences in the concentrations of ^{14}C in the plasma. Furthermore, with

Table I. Distribution of radioactivity 2 h after i.v. injection of sodium acetate-^{14}C with varying amounts of EDTA

Na_2Ca-EDTA (μmol)	Percentage of injected ^{14}C-dose [a] Plasma (1 ml)	Liver	Kidneys	Muscles [b]	Skeleton [c]	Labile skeletal fraction [d]
0	0.22 ± 0.01	3.63 ± 0.97	0.70 ± 0.07	9.65 ± 1.96	3.14 ± 0.32	1.12 ± 0.10
1	0.21 ± 0.03	4.57 ± 2.17	0.82 ± 0.06	9.87 ± 0.59	2.99 ± 0.18	1.02 ± 0.07
100	0.22 ± 0.02	3.09 ± 1.09	0.71 ± 0.11	8.66 ± 1.99	3.19 ± 0.12	1.02 ± 0.11
400	0.19 ± 0.02	2.28 ± 0.59	0.69 ± 0.15	7.62 ± 1.02	2.60 ± 0.37	0.91 ± 0.19

[a] Arithmetic means and 95%-fiducial limits. 5 rats per group. [b] Total muscle mass was assumed to equal 40% of body wt. [c] ^{14}C in one femur times 20. [d] Radioactivity released as $^{14}CO_2$ after treatment of femur with hydrochloric acid[2].

exception of the kidneys, there is no statistically significant influence of the lowest dose of EDTA on the accumulation of ^{14}C. With higher doses of EDTA, however, the retention of ^{14}C in the liver, kidneys and muscles decreases and regression lines may be calculated when plotting the experimental values against log EDTA dose. The estimated slopes of regression lines (Table II) in liver and muscles are virtually identical, while the ^{14}C content of the kidneys decreases more slowly. The linear terms of the regressions are significant. However, this correlation does not exist for the skeletal retention of ^{14}C, although this is significantly lower when 400 μmol instead of 100 μmol EDTA are added. Approximately one-third of the skeletal ^{14}C can be released by treatment with hydrochloric acid (Table I), irrespective of the amount of EDTA injected.

Our data are in favour of the above-mentioned hypothesis, i.e. they demonstrate a definite influence of EDTA on the distribution pattern of radioactivity after the injection of ^{14}C-labelled acetate. This might suggest the effect of EDTA on the metabolic turnover of acetate in soft tissues rather than the dose-dependent deposition of EDTA itself. The accumulation of ^{14}C in the skeleton is much less affected, and the loss of radioactivity in the bone after acid treatment indicating incorporation of ^{14}C into the labile carbonate pool in bone[5] is independent of EDTA. Since our knowledge of other than morphological effects of the chelating agents is extremely poor, further studies are required to show whether our observation on the effect of EDTA might be useful in evaluating the physiological and/or toxic action of the chelates[6].

Table II. Accumulation of ^{14}C in relation to log dose of stable EDTA

	Regression coefficient ± S.E.	Significance (P)
Liver	−0.844 ± 0.262	0.01
Kidneys	−0.053 ± 0.006	0.025
Muscles	−0.795 ± 0.247	0.01
Skeleton	−0.088 ± 0.071	0.30

Zusammenfassung. Die ^{14}C-Ablagerung nach i.v. Injektion von Ratten mit ^{14}C-markiertem Natriumacetat nimmt in Leber, Nieren und Muskeln mit steigender Zugabe (1–400 μmol) von gleichzeitig injiziertem Na_2[Ca-ÄDTA] ab. Hiermit wird ein bisher noch nicht beschriebener Effekt vom Chelatbildner nachgewiesen.

V. VOLF

Institut für Strahlenbiologie, Kernforschungszentrum, Postfach 3640, D-75 Karlsruhe 1 (Germany), 9 January 1970.

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Effect of Erythropoietin on the Activity of RNA Polymerase

The first molecular action of erythropoietin known until today is the early stimulation of the metabolic activity of a nuclear RNA of different characteristics[1,2]. These findings have suggested the idea that the primary site of action of the hormone may be at some level in the transcription stage[3]. To explore this possibility it seemed reasonable to study the activity of RNA polymerase under the influence of the hormone. Under these conditions the stimulation of the enzyme could be interpreted as the result of a direct control of the hormone on the RNA metabolism. This paper describes experiments that show the modifications produced in the activity of RNA polymerase in isolated nuclei from rat bone-marrow treated with erythropoietin.

Material and methods. Male rats of the strain A × C, weighing 150–170 g, were used. The cell nuclei were obtained from bone-marrow cells of normal and erythropoietin treated rats. The latter were collected from rats that received an i.v. injection of 5 units of erythropoietin. Bone-marrow cells were extracted from the femur and tibia and then homogenized in 10 vol. of cold 0.25M sucrose that contained $10^{-3}M$ $MgCl_2$. The homogenate

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