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MECHANISM OF THE ACTION OF DIISOPROPYL FLUOROPHOSPHATE

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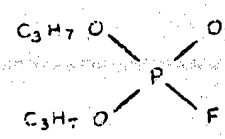
Submitted 17 August 1947

[Numbers in parentheses in the text refer to the appended bibliography.]

Conclusions on the role of acetylcholine in the transmission of a stimulus in nerve and neuromuscular synapses are based to a considerable degree on the study of the physiological effects of various substances with anticholinesterase actions. Physostigmine and prostigmine used in these physiological studies are such substances. The widely held opinion is that the effectiveness of these substances depends entirely on their inactivation of cholinesterase (1); the differences in their actions, such as the stimulative action of physostigmine and the depressive action of prostigmine on the central nervous system, as well as the differences in actions on the neuromuscular transmission of a stimulus are prone to be explained by the unequal penetrability of the 3-amine, physostigmine, and the 4-ammonium base, prostigmine (2,3).

A new and powerful anticholinesterase compound, diisopropyl fluorophosphate (DFP), has been intensively studied in recent years.

This compound irreversibly inactivates the positive and pseudo-cholinesterase both in vitro and in vivo in animals and is similar to physostigmine in action (4,5). Up to the present time there is no indication that DFP reacts in tissue ferments other than cholinesterase.



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The simple chemical structure and stability of DFP, its solubility in water and organic solvents, and the irreversibility of the inactivation of cholinesterase all make DFP an exceptionally valuable medium for the inactivation of enzymatic hydrolysis of acetylcholine in tissues. Actually, DFP has been used for this purpose in physiological research during the last two years on the transmission of an impulse through the nerve trunk (6-9), muscles (10), nerve synapses in insects (11), and reflex transmission through the central nervous system (12), as well as in clinical study (13).

It was discovered that the actions of these substances differed in some cases together with manifestations which are identical for DFP and physostigmine-prostigmine type preparations. Thus, DFP was found to be ineffective in myasthenia. DFP and physostigmine produced different effects on synaptic transmission in insects.

Further comparative research on the effects of DFP and physostigmine in various living tissues is of interest in this respect. In our research, we used the straight abdominal muscle of a frog, which is widely used for quantitative determination of acetylcholine. We studied the influence of DFP and physostigmine on the contractive reaction of the straight abdominal muscle to acetylcholine and to nonfissile cholinesterase carbocholine and choline.

Method

The experiments were conducted in May-June 1947 on Rana temporaries which had been kept for 4-6 days at 2-3 degrees C prior to the experiment. The isolated straight abdominal muscle was treated by the accepted method and immersed in a tall glass filled with Ringer solution. The upper end of the muscle was tied to a lightly loaded small lever with a ratio of the arms of 1:8. Air was constantly flowing through the solution. The process was recorded on a slowly moving kymographic cylinder which registered each contraction for 3 minutes. The preparations used in the experiments were poured into the glass in volumes not greater than 0.3-0.5 ml, each time to a total volume of the liquid of 10 ml.

Results

The treatment of muscles with DFP produces a sharp increase in their sensitivity to acetylcholine. The extent of this sensitivity depends on the duration of immersion and the concentration of the solution. The maximum effect produced after 20-30 minutes immersion in a concentration of $1\text{-}2:10^4$ DFP was approximately the same in a concentration of $2\text{-}3:10^6$ physostigmine. However, in the time required to wash away the sensitizing action of physostigmine the effect of DFP is not changed, and apparently this effect is irreversible.

The maximum effect of DFP is not strengthened by subsequent treatment with physostigmine in concentrations mentioned previously and, similarly, the maximum effect of physostigmine is not increased by further treatment with DFP. However, an additional treatment with either of these substances following an incomplete effect with the other produces a corresponding increase in their effects.

An unusual effect is observed in a DFP-treated muscle when treated with physostigmine in concentrations greater than that which gives a maximum sensitization, namely, $2\text{-}3:10^5$. Physostigmine in this concentration produces only an insignificant increase in the sensitivity of a fresh muscle. After treating with DFP, treatment with an identical concentration of physostigmine sharply depresses the reaction of the muscle to acetylcholine. (Figure 1). In some cases the muscle stops reacting to a quantity of acetylcholine several times greater than that which caused contraction in the beginning of the experiment. This depressing effect of physostigmine disappears after about 15-20 minutes washing, and the increased reactivity of the muscle which is characteristic of DFP is restored.

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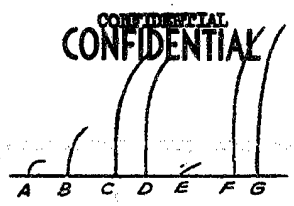

Figure 1

A - contraction of the straight abdominal muscle of a frog from 0.05 γ of acetylcholine; B - the same dose of acetylcholine after the ten minutes treatment with DFP $5 \cdot 10^{-5}$; C and D - the same dose of acetylcholine after a repeated treatment with DFP $1 \cdot 10^{-4}$; E - the same dose of acetylcholine after treatment with physostigmine $3 \cdot 10^{-5}$ for ten minutes; F and G - the same dose of acetylcholine after washing away the physostigmine.

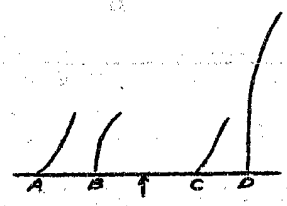

Figure 2

A - contraction of the straight abdominal muscle of a frog from 0.6 γ of carbocholine; B - from 0.1 γ acetylcholine; arrow - action of DFP $5 \cdot 10^{-5}$ for 25 minutes; C - carbocholine 0.6 γ; D - acetylcholine 0.1 γ.

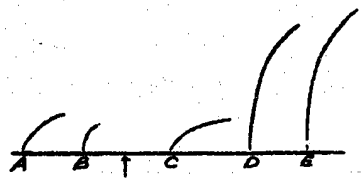

Figure 3

A - contraction of the straight abdominal muscle of a frog from 50 γ of choline; B - from 0.1 γ acetylcholine; arrow - action of DFP $5 \cdot 10^{-5}$ for 20 minutes; C - 50 γ choline; D and E - 0.1 γ acetylcholine.

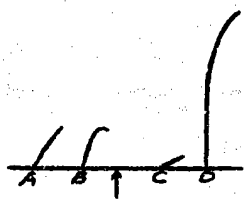

Figure 4

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A - contraction of the straight abdominal muscle of a frog from 0.6 γ of carbocholine; B - 0.3 γ acetylcholine; C - the same dose of carbocholine after treatment with physostigmine $2 \cdot 10^{-6}$; D - 0.3 γ acetylcholine.

Sensitization of the straight abdominal muscle after treatment with DFP is observed only in relation to acetylcholine. The reaction of the muscle to carbocholine and choline does not change (Figures 2 and 3). In contrast to DFP, concentrations of physostigmine which produce expressed sensitivity to acetylcholine depress the reaction of the muscle to carbocholine (Figure 4). It is interesting to note the difference in the contraction curves in the action of acetylcholine, carbocholine, and choline. In the first case the rapid initial rise slows down sharply, while in the action of carbocholine and choline the contraction increases slowly almost in a straight line, and the angle of slope of this line changes according to the dosage. These curves, each of which were recorded over a 3-minute period, do not register the maximum degree of contraction, but its speed. In order to obtain the same degree of contraction, acetylcholine, carbocholine and choline were used in concentration in a ratio of 1:2:500. A retardation in the speed of contraction and the absence of a definite maximum were observed in the reaction to acetylcholine in the muscle after it had been treated with DFP or physostigmine. It can be assumed that the contraction curve indicating the effect of acetylcholine reflects the interaction of two processes: the diffusion of acetylcholine in deeper layers of muscular fibers and its destruction which takes place in the tissues through cholinesterase. The increase in the contraction stops when these processes are equal to each other. In the action of carbocholine and choline on the muscle, the inactivation of these substances and the form of the contraction curve depends on the gradual diffusion of these substances within the muscle and the gradual implication of new fibers.

From our observations showing that DFP increases the effect of acetylcholine on the straight abdominal muscle but that carbocholine and choline do not, it is possible to conclude that the cause of the increased sensitivity depends on the inactivation of cholinesterase in the muscle. The same mechanism is the basis of the sensitizing action of physostigmine. Other observations (14, 15) indicate the combining of DFP and physostigmine in tissue by the same receptive groups. However, the action of physostigmine apparently is not limited by cholinesterase; this is indicated by the lowering of sensitization with an increased concentration of physostigmine and the sharp depressive action discovered in those cases where the physostigmine is applied after the DFP. Nachmansohn (16) shows that physostigmine retards not only cholinesterase, but also adenosinetriphosphatase. It is possible that in the destruction of cholinesterase by the preceding treatment with DFP, the action of physostigmine on the adenosinetriphosphatase is more sharply expressed. Our preliminary experiments showed that Mg.. has a sharply depressive action on the contraction of the straight abdominal muscle of a frog from acetylcholine and, according to some authors, is an inhibitor of adenosinetriphosphatase (17).

Thus, in using any substance having an anticholinesterase action, it is necessary to consider the possibility of its influence also on other processes taking place in the accomplishment of the reaction of a given tissue to acetylcholine. According to the view developed by Koshtoyants (18), synthesis and destruction of acetylcholine are only links in a complex enzymo-chemical system of development of a stimulus specific for each tissue. In the case of the contractive reaction of the skeletal muscle, the acetylcholine cycle is directly connected with the system of adenosinetriphosphoric acid - adenosinetriphosphatase. The dual character of the action of physostigmine illustrates this situation very well. On the other hand, the peculiar action of anti-cholinesterase preparations on different living tissues was shown by Kahlson and Uvnäs (19) from studies of contractive reaction to acetylcholine of the dorsal muscle of a leech, and the straight abdominal muscle and stomach muscles of a frog after treatment with physostigmine, quinine, and other substances which suppress the action of cholinesterase. These authors obtained a very confused picture, which is not explained by a single stopping of cholinesterase, and they presumed the existence of a nonspecific

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sensitization of tissue receptors to acetylcholine. These results, from our point of view, are explained by the fact that together with the sensitization connected with the stopping of one enzymatic system, a stimulating or retarding action on other processes is possible.

The DFP preparation, having the same expressed anticholinesterase action as physostigmine but distinguished from physostigmine in its relation to other enzymo-chemical systems, is a useful medium for explaining the role of acetylcholine in the transmission of stimuli and in the contractive action.

Bibliography

1. W. Feldberg, Physiol. Rev., 25, 596 (1945)
2. A. Schweitzer, E. Stedman, and S. Wright, J. Physiol., 36, 302 (1939)
3. K. Bernheim, Interaction of Drugs and Cell Catalysis, 1943
4. A. Mazur and O. Bodansky, J. Biol. Chem., 163, 261 (1946)
5. A. Mazur and O. Bodansky, ibid., 164, 271 (1946)
6. F. Crescitelli, G.B. Koelle and A. Gilman, J. Neurophysiol., 9, 241 (1946)
7. J.H. Bullock, H. Grundfest, D. Nachmansohn, M. A. Rothenberg, and K. Sterling, ibid., 9, 253, (1946)
8. J.H. Bullock, H. Grundfest, D. Nachmansohn, and M.A. Rothenberg, ibid., 10, 63 (1947)
9. J.H. Bullock, H. Grundfest, and D. Nachmansohn, ibid., 10, 11 (1947)
10. R. Couteaux, H. Grundfest, D. Nachmansohn, and M.A. Rothenberg, Science, 104, 317 (1946)
11. K.D. Roeder, N.K. Kennedy, and E.A. Tomson, J. Neurophysiol., 10, 1 (1947)
12. C. Heymans and R. Pannier, Proc Soc. Exp. Biol. and Med., 62, 229 (1946)
13. J.H. Comroe, J. Todd, and G.B. Koelle, J. Pharmacol. Exp. Therap., 87, 281 (1946)
14. R. Koster, ibid., 88, 139 (1947)
15. G.B. Koelle, ibid., 88, 232 (1947)
16. D.Nachmansohn, Currents in Biochem. Res., Interscl. Publ., N.Y. (1946)
17. M.N. Lyubimova and D. Pevzner, Biokhimiya, 6, 178 (1941)
18. Kh. S. Koshtoyants, Izv A.N. USSR, Ser.Biol., No 2, 170 (1945)
19. G. Kahlson and B. Uvnas, Skand Arch. Physiol., 78, 40 (1938)

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