THE PHARMACOLOGICAL CHARACTERISTICS OF THE CHOLINE REACTIVE BLOOD VESSEL SYSTEMS AND THE EFFECT UPON THEM OF SODIUM NITRITE AND BARIUM CHLORIDE*

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According to the views most widely held at present, the property possessed by sodium nitrite to weaken and by barium chloride to strengthen the contractions of smooth musculature is a result of its direct influence upon the muscle. However, it should be noted that this question has not been studied in recent years especially from the viewpoint of analyzing the basic biochemical processes underlying the act of smooth muscle contraction and studying those biochemical reactions which mediate the nerve impulse.

According to the literature [16, 20, 25], the essential action of the nitrites upon biochemical reactions consists in blocking those reactions which enable the organism to synthesize or utilize the adenotriphophoric acid which, according to present day concepts, is the basic source of the energy employed by the organism. It is known that adenotriphosphoric acid has to be present if these reactions are to take place and they are at the base of all muscular, including the smooth, contraction [4]. This does not warrant the conclusion that the nitrites act directly upon the muscle tissue itself. Thus brain tissue manifests a much greater sensitivity to lack of adenotriphosphoric acid than does muscle tissue [21] especially if this takes place during a period of oxygen deprivation. It should also not be forgotten that muscle tissue has a much greater capacity for the resynthesis of adenotriphosphoric acid than do other tissues as, for this purpose, aside from respiration, glycolysis may be utilized. In addition, muscular tissues use the energy derived from creatine phosphoric acid. Among the biochemical processes occurring in brain and nerve tissues attention should be drawn to the participation of adenophosphoric acid in the events of the cholinergic cycle, in particular the synthesis of acetylcholine [6, 27].

Description of the details of the influence exerted by barium chloride salts upon biochemical processes were not found in the literature available to us. Nevertheless, some facts speaking of their effect upon biochemical processes lying at the base of the activity of nervous tissue are known. Thus, attention is drawn to the weakening of intestinal response to barium chloride in the presence of thiamin avitaminosis [17]; the role played by the same vitamin B_1 in the normal activities of the nervous system, partially in the flow of the normal cholinergic processes, being well known. From the studies of A. I. Poskalenko [15], it can be seen that such fermentative poisons as fluorine or the cyanides, which upset carbohydrate metabolism, depress also the action of barium chloride and the influence of acetylcholine.

From this it may be assumed that barium chloride salts affect carbohydrate metabolism while those processes directly associated with the contraction of smooth musculature are related to the so-called adenylic cycle [4, 8].

Finally, it should be recalled that, according to the findings of N. V. Ermakova and G. G. Dyadyushi [3], preventing the access of barium chloride to the intramuscular portion of the innervation apparatus, markedly weakens the influence of barium upon frog striated muscle.

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All these considerations give ground for the supposition that nitrites and barium salts act upon smooth muscles primarily by influencing the nerve tonus regulating the smooth musculature, partially through the medium of influences effected with the aid of the mediation of acetylcholine.

The experimental verification of this supposition is the basis of the present study.

EXPERIMENTAL METHODS

Experiments were performed upon the liver vessels (of a frog) isolated by the method of V. I. Berezin. The livers were taken from pond frogs of the autumn collection as well as from freshly-caught spring frogs. The pressure under which the solutions were perfused through the liver vessels varied in the various experiments between 2.5 and 3.5 cm water column readings.

At first, it was necessary to clarify the nature of acetylcholine action and other cholinergic substances upon the frog liver vessels.

An analysis of experiments performed by numerous investigators [11,14,18,19], permits the following deductions: frog liver vessels are sensitive to substances of the acetylcholine group (acetylcholine, carbocholine, arecholine and pilocarpine) and also of the nicotine group (nicotine, lobeline and cytisine). Also active are choline-sensitizing substances e.g., physostigmine and proserine. All of these have a vasoconstricting action. Nicotine, pilocarpine and, partially, physostigmine, after a two-hour perfusion, and an initial vasoconstriction, dilate the liver vessels. Of the cholinelytic substances we studied atropine and platyphylline. Atropine even when perfused for a long time narrows the liver vessels removing however the constricting capacities of arecoline and carbocholine. Platyphylline widens the frog liver vessels; occasionally, when rinsing platyphylline out, some constriction of the vessels is observed.

Analysis of this data permits the supposition that the liver vessel net has present within it N (nicotine - sensitive) choline-reactive systems, as classified by S. V. Anichkov [1]. In favor of this theory is the two phase action of nicotine as well as the sensitivity of the frog liver vessels to lobeline and cytisine. Also, attention is drawn to the two phase action of pilocarpine which is similar to its effect on the N choline reactive system in the cardiac vagal ganglion [9].

In order to verify that the vasodilating action of platyphylline is associated with a blockade of the M (muscarine sensitive) choline reactive system, we investigated the effect exerted by this substance upon the action of acetylcholine. Altogether 15 tests were performed. Marriott vessels were used to contain the perfusion fluid—a clear solution of normal Ringer's as well as a bitartrate solution of platyphylline. With the aid of a syringe, there was added to the perfusing fluid 0.2 cc of a 1:1000 solution of acetylcholine.

Platyphylline did not remove the acetylcholine action from the frog liver vessels. Thus, when introduction of acetylcholine during perfusion of the liver with Ringer's solution diminished, on the average, the number of drops flowing out of the liver veins by 63%, against a preliminary rinsing of the liver with platyphylline (1:2000), when the dilatation of the vessels had reached a stable extent, under the influence of acetylcholine the number of drops decreased an average 83%.

The ability of atropine to remove the vasoconstricting effects of arecoline and carbocholine can be completely attributed to the influence exerted by atropine upon the N choline sensitive vessel system, at least as far as there is, for example, data on the ability of atropine to block them in the sympathetic ganglia [23,24]. It is also known that arecoline can stimulate also the N choline sensitive systems as, e.g., striated muscles [2]. The ability of atropine to act like acetylcholine in constricting the frog liver vessels also is contrary to the concept of it blocking the M choline sensitive systems and is more to be explained by the anticholinesterase action of the atropine which is a property more or less common to all substances capable of competing with acetylcholine because of their choline sensitive structure [5]. This anticholine esterase action may be the reason for platyphylline reenforcing the acetylcholine action in our experiments. We have already pointed out that, according to the literature, typical anticholinesterase substances such as physostigmine and proserine* also exert a vasoconstricting influence upon frog liver vessels. At this point it should be noted that, according to many opinions [7, 10, 11, 12], the regulation of vasomotor tone, aside from central influences, is mediated by a nervous mechanism present in the vessel walls. In this manner, the analysis of the literature and our own data leads us to the conclusion that the choline reacting vessel systems in the frog liver belong to the nicotine sensitive group.

* Russian trade name.

According to Japanese investigators [22], acetylcholine exerts a nicotine like action upon the vessels in the frog rear limbs.

In order to determine whether the vasodilating action of sodium nitrite is a result of a blockade of the choline reactive systems, we utilized the antagonism between proserine and the cholinelytic action of certain pharmacological substances. As is known, this action is regarded as a manifestation of the anticholinesterase influence of proserine as a result of which the competing relations of the spontaneously forming acetylcholine and cholinelytic agents within the tissues are altered in their similarities to their choline sensitive structure: the sensitivity of the latter becoming restored.

First, we had to investigate whether we could demonstrate this antagonism in our experimental object. We studied the influence of proserine (1:4000 and 1:2500) upon vasodilation produced by large concentrations of the nicotine like substance anabasine (1% and 1.5%). It has been established that large concentrations of nicotine like substances block N choline sensitive systems.

As can be seen from Table 1, in the frog liver vessels, proserine manifests the capacity of removing the blockade of the N choline reactive systems.

TABLE 1

Influence of Proserine Upon the Vasodilating Action of Anabasine (experiment performed April 2, 1952)

Time from start of exp. (min)	Perfusion fluid	Drops per minute
0—6	Normal Ringer solution	51. 51. 51. 51. 51. 51
7—15	Anabasine 1 : 100	55. 54. 55. 56. 57. 58. 58. 60. 62
16—21	Anabasine 1 : 100 + Proserine 1 : 2500	59. 57. 51. 49. 49. 49
22—101	Rinsing with Ringer solution	—
102—105	Ringer solution	50. 51. 50. 50
106—120	Anabasine 1 : 100	51. 51. 52. 52. 54. 53. 55. 55. 56. 58. 57. 57. 61. 60

In the succeeding 40 experiments we investigated the influence of proserine (1:4000 and 1:2000) upon the vasodilating action of sodium nitrite (1:20,000 and 1:10,000). The protocol of one such experiment of this series is shown in Table 2.

As A. A. Nikulin [14] has shown, at a pH of 8.3 sodium nitrite constricts the frog liver vessels. But as we have shown in our experiments neither nitrite nor its mixture with proserine altered the reaction of Ringer's solution medium. Hence, the vasodilating action of sodium nitrite must be associated with a blockade of the N choline reactive structures in the frog liver vessels.

To analyze the effect of barium chloride upon the frog liver vessels, we decided to observe the influence dimedrol had upon the vasoconstricting power of barium as dimedrol had been shown by M. D. Mashkovsky and S. S. Liberman [13] to have the ability of blocking N choline reactive systems. To confirm this ability of dimedrol to block the N choline reactive systems in the frog liver vessels we decided to test the influence of dimedrol upon the vasoconstricting power of anabasine sulphate (10 experiments): taking for this purpose 0.5 cc of a 6% anabasine solution, prepared in Ringer solution and introduced directly into the stream of the perfusing fluid.

It became apparent that dimedrol dilated the frog liver vessels. The vasoconstricting action of anabasine was not manifested if the liver vessels had been first perfused by dimedrol which dilated the liver vessels to a constant width.

To convince ourselves that the vasodilating and cholinelytic action of dimedrol is associated with its

* Russian trade name.

influence upon the choline reactive systems and is not a direct effect upon the biochemical processes underlying the act of muscle contraction, in this series of experiments, as with those done with sodium nitrite, we tested in 10 experiments the influence of proserine upon the vasodilating action of dimedrol (Table 3).

We see that proserine removes the dimedrol action; it follows that the vasodilating effect of dimedrol is associated with a blockade of the N choline reactive systems in the vessels of the frog liver.

After this we investigated the influence of dimedrol upon the vasoconstricting action of barium chloride (20 experiments). 0.5 cc of a 6% solution of barium chloride introduced into the perfusion fluid (Table 4).

TABLE 2

Influence of Proserine Upon the Vasodilating Action of Sodium Nitrite (February 22, 1952)

Time from start of exp. (min)	Perfusion fluid	Drops per minute
0—5	Normal Ringer	42. 41. 42. 41. 42
6—26	sodium nitrite 1: 10 000	42. 43. 43. 44. 46. 46. 46. 46. 47. 48. 47. 49. 49. 48. 49. 50. 51. 51. 50. 51. 51
27—32	Sodium nitrite 1: 10 000 + Proserine 1 : 2 000	51. 49. 44. 38. 37. 35
33—67	Rinsing with Ringer	—
68—71	Ringer solution	42. 41. 41. 41
72—82	Sodium nitrite 1 : 10 000	41. 42. 43. 46. 47. 48. 49. 49. 50. 50. 50
83—94	Sodium nitrite 1: 10 000 + + proserine 1 : 2 000	49. 49. 46. 43. 40. 38. 37. 36. 32. 27. 23. 15
95—136	Rinsing with Ringer solution	—
137—140	Ringer solution	35. 35. 36. 35

TABLE 3

Influence of Proserine Upon the Vasodilating Action of Dimedrol (experiment performed January 27, 1953)

Time from start of exp. (min)	Perfusing fluid	Drops per minute
0—5	Normal Ringer solution	46. 45. 45. 46. 45
6—10	Dimedrol 1 : 500	46. 45. 45. 45. 45
11—39	Same	—
40—43	» »	52. 52. 52. 52
44—49	Dimedrol 1 : 500 + Proserine 1 : 2 000	48. 45. 40. 39. 39 37
50—79	Rinsing with Ringer	—
80 - 83	Ringer solution	45. 46. 46. 46

These experiments show that dimedrol blocks the vasoconstricting action of barium chloride. It follows that the action of barium chloride is associated with stimulation of the N choline reactive systems in the vessels of the frog liver.*

TABLE 4

Influence of Dimedrol Upon the Vasoconstricting Action of Barium Chloride (experiment performed February 5, 1953)

Time from start of exp. (min)	Perfusing fluid	Drops per minute
0—4	Normal Ringer solution	43. 43. 43. 43
5—14	Barium chloride	52. 44. 41. 38 37 38. 38. 39. 38 39
15—18	Dimedrol 1 : 500	38. 40. 39. 38
19—47	Same	--
48—51	» »	47. 48 48. 48
52—64	Barium chloride	48. 48. 49. 48. 48 48. 48 48. 48. 48 48. 48 48
65—130	Rinsing with Ringer solution	—
131—134	Ringer solution	43. 42. 42. 42
135—145	Barium chloride	46. 41. 39. 38. 38. 38. 37. 37. 35. 36. 35

EXPERIMENTAL RESULTS

Pharmacological analysis shows that vasomotor tone regulation in the frog liver vessels is associated with cholinergic mechanisms. The choline reactive vessel systems in the frog liver belong to the nicotine sensitive group (S. V. Anichkov classification). The vasodilating action of sodium nitrite appears to be associated, apparently, with the influence of this substance upon cholinergic processes and not with a direct interference with the chemodynamics of the smooth blood vessel musculature as has been supposed.

The same remarks apply to the vasoconstricting action of barium chloride.

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* In the same year of 1954 when we reported our studies, Levy and Michel-Ber [26] showed that barium chloride acts on the bowel in a ganglionic manner.

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