Effect of Neostigmine on the Tissue Concentration of Antibiotic in Streptomycin Treated Rats

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Increased streptomycin levels in rat skeletal muscle tissue were observed after the administration of neostigmine methylsulfate alone and in combination with atropine. Acetylcholine and methacholine bromide were administered to determine if the increase in antibiotic concentration was due to vasodilation or to some other action of the anticholinesterase.

THIS AUTHOR (1–3) has reported both a potentiation of activity and an increase in the rate of tissue penetration of some agents which have been administered after cholinesterase inhibitors. Similarly, Greig (4) has noted an increased barbital concentration in mouse brain when the barbiturate was administered shortly after an anticholinesterase agent. The purpose of this investigation was to ascertain the concentration of streptomycin in skeletal muscle and blood serum of rats which received the antibiotic after the administration of either neostigmine methylsulfate alone and in combination with atropine sulfate or the choline esters—acetylcholine and methacholine bromide.

Streptomycin levels in muscle extracts and blood serum were determined by a bacteriological cup method. A standard curve was obtained after the method of Grove and Randall (5), with reference standards from 0.1 to 10 mcg. The curve was then used to determine the streptomycin level in the rat tissue.

EXPERIMENTAL

Procedure for Determining the Streptomycin Level in Rat Tissue (I).—Albino rats used in this study were selected without regard for age, weight, or sex. Control and experimental animals were studied concurrently in each procedure.

The experimental animals were administered 0.0004 mg./Gm. neostigmine subcutaneously. Thirty minutes after the injection of the cholinesterase inhibitor into the experimental animals, 40 mg./Kg. of streptomycin was administered intramuscularly into the right rear thighs of both the control and experimental animals.

Three hours after the administration of the streptomycin, the animals were etherized and sacrificed by severance of both common carotid arteries, whereupon the blood was collected in centrifuge tubes and refrigerated. The skin was then removed from the left rear leg and the muscle tissue removed. After washing the tissue to remove any remaining blood,[1] the fascia and fat were removed and 20 Gm. of the remaining muscle tissue added to 100 ml. of the cold phosphate buffer,[2] and homogenized for 15 minutes at high speed in a Waring Blendor. The homogenate was then diluted to 200 ml. with cold phosphate buffer to be used for testing.

Three cups on each of three agar plates to be used in the test were filled with 0.2 ml. of the homogenate and the other 3 cups filled with 0.2 ml. of a known concentration of streptomycin, 1 mcg./ml. of tissue extract, as a control. The plates were covered and incubated for 18 hours at 37° at which time the diameters[3] of the inhibitory zone for each cup of the homogenate and control were noted. The readings were then corrected to the 1 mcg./ml. reference point by the same procedure used in establishing the standard curve and the corrected zone measurements compared to the standard curve in order to interpret the number of micrograms of streptomycin per milliliter in the experimental test tissue homogenates. Because a dilution factor of the tissue was involved, 20 Gm. in 200 ml., the figures obtained from the standard curve were multiplied by 10 and the milligram concentration of streptomycin per gram of tissue was reported.

The Determination of the Effects of the Cholinesterase Inhibitor on the Serum Levels of Streptomycin in Rats (II).—The blood collected was allowed to clot in the refrigerator; the clot was then loosened with a sterile loop and the tube centrifuged at high speed about 25,000 r.p.m. for 7 minutes. After centrifuging, the serum was pipetted off and diluted from 1 part serum up to 4 parts with phosphate buffer. The streptomycin concentration of the serum was then determined by the same method as the antibiotic concentration of the muscle tissue, using 0.2 ml. of 10 mcg./ml. solution of streptomycin in the control cups as the control. The readings of the zones of inhibition and corrections were made after the same method used in part I, but using the 10 mcg./ml. reference point as the correction standard.

The Determination of the Effects of Atropine on

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[1] The wash liquid was the same phosphate buffer that was used for the dilution of the extract—dibasic potassium phosphate, 16.73 Gm. and monobasic potassium phosphate, 0.523 Gm. per 1000 ml. adjusted to pH 8.

[2] Cold phosphate buffer was used since the liquid in the blender had a tendency to heat up, thereby destroying part of the streptomycin activity.

[3] Since the zones of inhibition were irregular, six different measurements of the diameter for each zone were made and the average recorded as the diameter of the inhibitory zone.

the Streptomycin Levels in Neostigmine-treated and Untreated Rats (III).—Experimental and control animals were prepared and medicated in the same manner as in part I, except that the controls received 8 mg. of atropine sulfate subcutaneously 30 minutes prior to streptomycin and the experimental animals received 8 mg. of atropine sulfate subcutaneously with the neostigmine prior to streptomycin. Three hours after the administration of streptomycin, the animals were sacrificed and the muscle tissue and blood serum used for the determination of the streptomycin level as in parts I and II. The readings of the zones of bacterial inhibition, and corrections, were made as in parts I and II.

The Determination of the Effects of Methacholine and Acetylcholine on the Streptomycin Level in Rat Tissue (IV).—A vasodilator, methacholine bromide 0.05 mg./Kg. intramuscularly, was given to albino rats 15 minutes prior to 40 mg./Kg. of streptomycin. After 3 hours, the animals were sacrificed and the blood serum and muscle tissue collected and tested as in parts I and II above. Streptomycin tissue levels were determined and corrected for reporting as described above.

When acetylcholine was administered with neostigmine at a dose level of 0.05 mg./Kg., all animals succumbed, but by decreasing the acetylcholine dose to 0.01 mg./Kg. with neostigmine prior to streptomycin, the animals survived. The muscle tissue and blood serum in this group were checked as above.

RESULTS AND DISCUSSION

Streptomycin Concentration in Rat Tissue-Controls.—Six rats were given streptomycin 40 mg./Kg. intramuscularly; after 3 hours the animals were sacrificed and the blood and muscle tissue checked for antibiotic activity by the cup method.

The homogenate of the muscle tissue, 1:10 dilution, gave no inhibitory zones, indicating that the concentration of streptomycin was less than 0.005 mg. per gram of tissue by this method. Blood serum levels were checked and found to be, for the average of the six animals, 0.0402 mg./ml. These findings were recorded to be used as the basis of comparison for animals receiving prior medications.

Streptomycin Concentration in Tissue and Blood Serum of Rats Pretreated with Neostigmine.—Neostigmine 0.4 mg./Kg. was given subcutaneously to six rats 30 minutes prior to 40 mg./Kg. of intramuscular streptomycin. Three hours after streptomycin administration the animals were sacrificed; the blood and tissue were collected and tested for antibiotic activity as described above.

The muscle tissue showed streptomycin activity equal to 0.0159 mg./Gm. which was more than the controls, since the controls showed less than 0.005 mg. per gram. The serum level of streptomycin as determined in these tests showed only 0.0245 mg./ml., a 0.0157 mg./ml. decrease from that of the control animals.

Streptomycin Concentration in Rats Premedicated with Atropine and Neostigmine.—Eight milligrams of atropine sulfate plus neostigmine methylsulfate were administered 30 minutes prior to 40 mg./Kg of intramuscular streptomycin to six rats. Three hours after the streptomycin administration, the animals were sacrificed, the blood and muscle tissue collected and tested as above to determine the antibiotic activity.

One group of plates with muscle tissue of one animal showed no inhibition beyond the cups, but in the other five there were clearly defined zones of inhibition which gave an average reading of 0.0123 mg. of streptomycin per gram of muscle tissue. The blood serum level gave an average of 0.0299 mg. per ml for the six animals tested. These findings were similar to those for neostigmine alone.

Atropine 8 mg. per animal is a dose of atropine that should prevent the vasodilating effects of neostigmine, even though the rat is quite tolerant to the belladonna alkaloids. Because the administration of atropine did not change appreciably the tissue levels of streptomycin in the above animals, it appears evident that the increase in tissue levels of the antibiotic in neostigmine pretreated rats is not primarily due to vasodilation.

Streptomycin Concentration in Tissue of Rats Pretreated with Atropine Sulfate.—Six rats of varying ages, weights, and sex were administered subcutaneously 8 mg. of atropine sulfate 30 minutes before streptomycin 40 mg./Kg. intramuscularly. Three hours after the streptomycin injections, the animals were sacrificed and the blood and tissue collected for testing.

Five of the animals showed concentrations of streptomycin too small to read by this method, *i.e.*, less than 0.005 mg. per gram of tissue. One animal, however, showed slight inhibitory zones about the test cups, but the 1 mcg./ml. reference cups were much below the average which increased the corrected reading to a point that it could be estimated by extending the slope of the standard curve. However, the concentration of the antibiotic was still below 0.005 mg./Gm.

The blood serum level of streptomycin in the atropinized animals was 0.0216 mg./ml., which was less than the control but near that for animals pretreated with neostigmine alone or neostigmine and atropine combined.

Streptomycin Concentration in Rats Pretreated with Methacholine and Acetylcholine.—Methacholine bromide 0.05 mg./Kg. was administered to 4 rats subcutaneously and followed immediately by streptomycin 40 mg./Kg. intramuscularly. After 3 hours, the tissue and blood serum were collected from the sacrificed animals, as above, and tested for streptomycin activity.

The muscle tissue in methacholine pretreated rats showed a tissue level of 0.0076 mg./Gm. and the blood serum 0.02727 mg./ml. The muscle tissue antibiotic level was slightly more than the controls, and the blood serum level was much less than the serum level of the controls, but about the same as that of neostigmine treated and atropinized animals in this study.

Acetylcholine bromide 0.05 mg./Kg. was administered to four rats subcutaneously, followed immediately by streptomycin 40 mg./Kg. After 3 hours the animals were sacrificed; the tissue and blood serum collected and tested as above to determine the antibiotic level.

The muscle tissue showed a tissue level of streptomycin of 0.0068 mg./Gm. and the serum 0.0328 mg./ml. which was about the same as metha-

STREPTOMYCIN IN RAT TISSUE[a]

	Concn., Blood Serum, mg./ml.	Concn., Skeletal Muscle, mg./Gm.
Streptomycin alone	0.0402	Less than 0.005
Streptomycin and neostigmine	0.0245	0.0159
Streptomycin, neostigmine, and atropine	0.0299	0.0123
Streptomycin and atropine	0.0216	Less than 0.005
Streptomycin and methacholine	0.0272	0.0076
Streptomycin and acetylcholine	0.0328	0.0068

[a] The above was determined from tissue obtained from animals three hours after administration of 40 mg./Kg. of the antibiotic.

choline for the tissue, but a higher level for the serum.

This study of the effects of methacholine and acetylcholine on the tissue levels of streptomycin indicates that vasodilation produced by these agents did not cause as appreciable a rise in the tissue level of the antibiotic as does neostigmine alone, or in combination with atropine. If vasodilation were the only factor involved in the increase in the tissue level of streptomycin brought about by the neostigmine, methacholine and acetylcholine should have given an increase in the tissue level of the antibiotic equal to that produced by neostigmine.

SUMMARY AND CONCLUSION

The cup method using agar plates seeded with *B. subtilis* was used to determine the streptomycin concentration of the tissue and blood serum of rats receiving 40 mg./Kg. of streptomycin. Additional tissue and blood levels of streptomycin were determined on rats premedicated with neostigmine, neostigmine and atropine combined, atropine, methacholine, or acetylcholine.

Animals premedicated with neostigmine and neostigmine plus atropine, showed tissue levels of 0.0159 mg./Gm. and 0.0123 mg./Gm., respectively, which represented increases over the controls (streptomycin level less than 0.005 mg./Gm.). Also with the increased tissue level there was a decrease in the blood serum level.

Attempts to increase the rat tissue levels of antibiotic by the use of the vasodilators, methacholine and acetylcholine 0.05 mg./Kg., resulted in tissue levels of 0.0076 mg./Gm. and 0.0068 mg./Gm., respectively.[4] These tissue levels were slightly greater than the controls, but much less than the levels in neostigmine, or neostigmine and atropine combined, pretreated animals.

Atropine, 8 mg. per animal, failed to block the tissue level increases of streptomycin produced by neostigmine, although this dose of atropine should have blocked any vasodilating activity of neostigmine.

The observed increases in the rat muscle tissue level of streptomycin must be some action other than that of producing vasodilation by the action of the endogenous acetylcholine that accumulates with neostigmine administration.

From this investigation and previous studies by other authors,[5] it appears that some of the inhibitors of cholinesterase (6) increase the absorption and penetration of certain agents into tissue. Because neostigmine affected increases in the tissue levels of streptomycin, it would appear that it increases the permeability of the skeletal muscle to the antibiotic.

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[4] The above concentrations of streptomycin in methacholine and acetylcholine treated rats is for only half of the test animals since 50% showed tissue concentration less than 0.005 mg./Gm.

[5] Increases in streptomycin penetration in the CSF of dogs reported to the Scientific Section, A.PH.A., Washington, D. C. meeting, 1960.