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A Synergism of Physostigmine and Strychnine.

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The experiments of Feldberg, Minz and Tsudzimura[1] on the mechanism of the nervous discharge of epinephrine suggest the possibility of influencing the liberation of this hormone through the synergistic action of appropriate drugs If, as outlined in their theory, splanchnic stimulation results in the production of acetylcholine which in turn stimulates the adrenal medulla to discharge epinephrine, drugs capable of effecting splanchnic stimulation will act synergistically with drugs capable of prolonging the action of acetylcholine. Employing strychnine[2] and physostigmine,[3] as representatives of the aforementioned drug types, we have demonstrated in unanesthetized rats a synergistic hyperglycemic action, presumably due to the liberation of epinephrine since the effect disappeared when the adrenals were demedullated. In the quantities employed the drugs did not produce objectionable systemic effects.

Methods. The data were obtained by superimposing the actions of physostigmine and strychnine on a glucose tolerance test[4] standardized for the rat. Only male rats of the Wistar strain ranging in age from 93 to 219 days were used. The diet was uniform.

Fifteen to 16 hours subsequent to the removal of the food, the test glucose, 3.5 g per kg in a 10% solution, was injected intraperitoneally. Blood samples, sufficient for measuring 0.2 cc were milked from the tail before the glucose injection and ½, 1, 2, 3, and 5 hours after.

"True" sugar was determined by the Shaffer-Hartmann-Somogyi method[5] on blood filtrates prepared by the zinc hydroxide precipitation procedure.[6] Newcomer hemoglobin determinations indicated that the blood concentration changes were not sufficient to justify a correction of the blood sugar values.

Test routine. The data were obtained on 22 rats each subjected to the glucose tolerance test under the following conditions: (1) no

1 Feldberg, W., Minz, B., and Tsudzimura, H., *J. Physiol.*, 1934, **81**, 286.

2 Stewart, G. N., and Rogoff, J. M., *J. Pharm. and Exp. Ther.*, 1919, **13**, 95.

3 Stedman, E., and Stedman, E., *Biochem. J.*, 1931, **25**, 1147.

4 Cole, V. V., Harned, B. K., *Endocrinology*, 1938, **23**, 318.

5 Shaffer, P. A., and Somogyi, M., *J. Biol., Chem.*, 1933, **100**, 695.

6 Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

drug; (2) with strychnine; (3) with physostigmine; (4) with physostigmine and strychnine. Subsequently, the adrenals were demedullated and procedures (1), (3), and (4) repeated. The routine was limited to 6 tolerance tests per day; 2 of the rats received strychnine, 2 physostigmine, and 2 strychnine and physostigmine. Although 6 controls (no drug) were made on a single day, they were distributed at regular intervals throughout the experimental period. According to data of the authors,[4] the glucose tolerance of Wistar rats is constant over a much larger age range than that involved in the present study. Tests on individual animals were spaced 10 to 14 days apart. Immediately after the 1 hour blood had been taken the drug was injected subcutaneously and the difference between the blood sugar values obtained with and without the drug measured the effect of the drug. The ½ hour and the 1 hour bloods served as added controls on the condition of the animals.

Throughout the experiments the drugs and the glucose were dissolved in distilled water immediately before their injection. The physostigmine and the strychnine were administered in the following concentrations and quantities: (1) strychnine sulfate (U.S.P. Merck), 0.52 mg per kg in a 0.035% solution; (2) physostigmine salicylate (U.S.P. Merck), 0.10 mg per kg in a 0.010% solution. The physostigmine used in the experiments was obtained from one ampoule, kept dry in a desiccator.

Results. In the concentrations employed the drugs rarely gave external evidence of activity. The quantity of physostigmine was selected because it appeared to be the largest amount just failing to produce salivation and of the 44 injections of this drug alone and in conjunction with strychnine, salivation was observed in only 2 instances. There was no cathartic action, no muscular twitching, and, according to palpations at significant intervals, no change in heart rate. Comparing the blood sugar values obtained after physostigmine with those of the corresponding control periods (Table I), the elevation produced by the drug amounted to 41 mg % on the first hour, 35 mg % on the second hour, and 20 mg % on the fourth hour.

The amount of strychnine administered was approximately the smallest quantity producing an elevation of the blood sugar. By the fourth hour subsequent to the injection, the strychnine effect apparently had disappeared, but on the first and second hours the elevation averaged 6 mg %. The chance that this figure is significant is 9 to 1. Probably, the synergism would have been magnified if a larger quantity of strychnine had been chosen.

TABLE I.

Effect of Physostigmine and Strychnine on Glucose Tolerance of Normal and Demedullated Rats.

	No. of animals	Fasting*	Hr after glucose injection: ½	1	2	3	5
			Hr after drug injection:		1	2	4
		"True" blood sugar in terms of glucose per 100 cc. of blood					
		mg	mg	mg	mg	mg	mg
No drug	22	66 ± 1.0	170 ± 3.2	136 ± 2.7	113 ± 1.4	112 ± 0.8	110 ± 0.8
Strychnine	21	66 ± 1.0	172 ± 3.3	134 ± 2.2	119 ± 2.0	118 ± 2.3	110 ± 2.6
Physostigmine	22	64 ± 0.7	171 ± 3.2	137 ± 2.8	154 ± 3.4	147 ± 2.9	130 ± 5.0
Physostigmine and strychnine	22	66 ± 0.8	171 ± 3.2	135 ± 2.9	174 ± 5.1	173 ± 5.9	134 ± 4.1
		After adrenal demedullation					
No drug	18	67 ± 0.9	171 ± 2.8	135 ± 3.1	107 ± 3.1	92 ± 3.1	89 ± 2.7
Physostigmine	18	71 ± 0.7	166 ± 3.8	121 ± 2.7	108 ± 2.0	97 ± 2.4	68 ± 2.4
Physostigmine and strychnine	18	68 ± 0.9	157 ± 3.4	118 ± 1.7	107 ± 2.0	85 ± 2.8	60 ± 4.0

*Food withdrawn 15 hours before control blood.

Glucose was administered intraperitoneally, 3.50 g per kg of body weight. The drugs were injected subcutaneously immediately after the 1-hour bloods. When 2 drugs were administered, they were given separately. The drug dosages per kg were: physostigmine salicylate, 1.0 cc of a 0.01% solution; strychnine sulfate, 1.5 cc of a 0.035% solution.

When the physostigmine and strychnine were injected simultaneously the synergistic rise in the blood sugar amounted to 14 mg % on the first hour, 20 mg % on the second hour, and 4 mg % on the fourth hour (Fig. 1). On a percentage basis the synergistic action increased the hyperglycemic effect of the strychnine 230% on the first hour, and 330% on the second hour, or it increased the summed effects of strychnine and physostigmine 29% and 47% respectively on the first and second hours. While the data for the fourth hour subsequent to the drug injection do not permit a definite conclusion, they contain the suggestion that physostigmine has demonstrated a strychnine action not detectable in its absence.

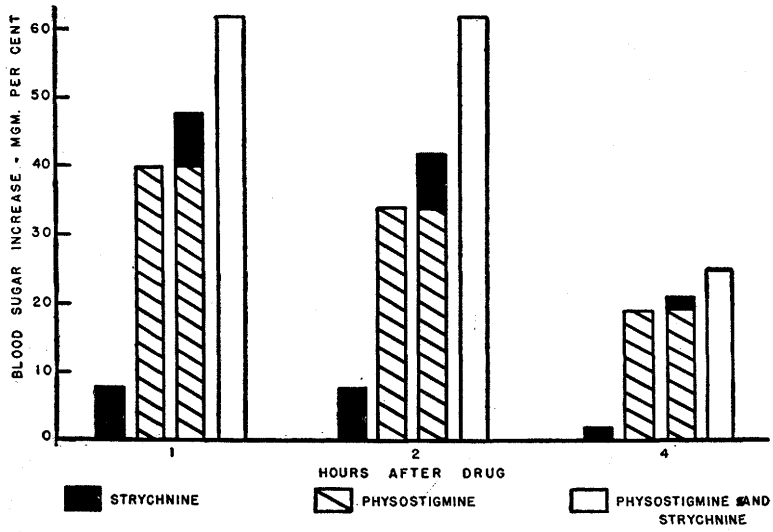

FIG. 1.

Hyperglycemic Synergism of Physostigmine and Strychnine.

The drugs were administered subcutaneously in the following dosages per kg: physostigmine salicylate, 1.0 cc of a 0.01% solution; strychnine sulfate, 1.5 cc of a 0.035% solution.

Supplementing the statistical analysis of the mean values given in Table I, the range of the blood sugar variations provides additional evidence of the synergism. It is significant that, although the boundaries for the range of the blood sugar variations of strychnine alone closely approximate those of the control, without drugs, the boundaries for the combination of strychnine and physostigmine are impressively above the corresponding limits of physostigmine alone.

The quantities of physostigmine and strychnine employed are not considered optimal for the synergistic demonstration since they were selected as representing definite hyperglycemic doses free from objectionable side reactions in the rat.

After demedullation of the adrenals by Evans' technic[7] the hyperglycemic action of the drugs almost, if not entirely, disappeared (Table I). The effect of the drugs may be evaluated by comparing the blood sugar averages with and without the drugs at a given time or by comparing the progressive changes in the control series with those in the drug series. With the unoperated rats the 2 methods give identical results but with the demedullated animals the first method shows no hyperglycemia from the drugs while the second method indicates a questionable rise in the blood sugar. Using the second method, with the value of the blood sugar immediately before the drug injection as the point of reference, physostigmine and strychnine produce after one hour a hyperglycemia of 18 mg % in the demedullated rats as compared with 62 mg % in the unoperated animals and after 2 hours, 10 mg % in the operated but 62 mg % in the unoperated. Four hours subsequent to the drug injections the demedullated rats showed a hypoglycemia of 12 mg % while the unoperated rats showed a hyperglycemia of 25 mg %. Control experiments on the demedullated rats were made 25 and 60 days after demedullation. The glucose tolerance remained essentially unchanged. During the interval the drugs were administered. The quantitative absorption of the sugar from the intraperitoneal injection did not differ from that in the unoperated animals.

Conclusions. Physostigmine and strychnine administered to normal rats in doses of 0.10 and 0.52 mg per kg respectively, exerted a statistically significant, hyperglycemic synergism. The synergistic action increased the mean of the maximal hyperglycemia of strychnine 330% and the sum of the means of the maximal actions of physostigmine and strychnine 47%. The quantities of the drugs employed did not produce objectionable systemic effects. After demedullation of the adrenals the drugs lost their hyperglycemic action.

[7] Evans, G., *Am. J. Physiol.*, 1936, **114**, 297.