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THE PRESSOR EFFECT OF PEPTONE IN THE RAT

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Peptone normally causes a rise of blood pressure in rats. Since the pressor response was not abolished or even diminished by adrenalectomy, it could not have been caused by a release of pressor substances from the adrenal glands. The group of drugs (dibenamine, dihydroergotamine, ergotamine, tolazoline, nicotine and hexamethonium) which antagonize or block the peptone-induced pressor action suggests that the pressor effect may be secondary to a release of sympathin by the stimulation of sympathetic ganglia. Since it was not possible to duplicate the pressor response to peptone by either 48/80 or histamine, the hypothesis that peptone acts by liberating histamine can be ruled out.

In the course of experiments on the effect of peptone on the mast cells of the rat, it was found that, following a slight fall, a rise in blood pressure appeared after an intravenous injection of peptone (Beraldo and Mota, 1953).

It has long been known that a rise in blood pressure may follow the initial fall after an intravenous injection of either histamine or pilocarpine into a spinal cat. The appearance of a secondary rise after injection of those drugs has until recently been generally assumed to be due to liberation of sympathin from the adrenals (Dale and Laidlaw, 1912; Burn and Dale, 1926). This does not fully account for the secondary rise since Root (1951) found that the secondary rise caused by pilocarpine was unaffected by adrenalectomy, whereas Slater and Dresel (1952) observed the persistence of a small secondary histamine rise after adrenalectomy. Recently, Trendelenburg (1955) investigating the factors involved in the appearance of the secondary rise in blood pressure after intravenous injections of histamine and pilocarpine concluded that, while the histamine rise is mainly due to liberation of sympathin from the adrenal gland and only to a minor degree to general stimulation of sympathetic ganglia, the pilocarpine rise, on the other hand, is mainly due to the ganglionic actions of this substance and is thus similar to the histamine rise in the adrenalectomized cat.

The rise in blood pressure which follows the injection of peptone could not be accounted for by the histamine usually present in the peptone, since the peptone used was freed of histamine by treatment with permutit. However, as peptone is a potent histamine releaser (Dragstedt, 1937; Feldberg and O'Connor, 1937; Rocha e Silva, Teixeira and Andrade, 1946), it might be possible that the sympathin could be released by histamine liberated from tissues of the rat.

On the other hand the hypothesis that the liberation of other pressor substances from the rat tissues might account for the rise of blood pressure has also been put forward. Both hypotheses have been tested in the experiments reported in this paper.

METHODS

Male Wistar rats weighing 200 to 400 g. were used. The animals were anaesthetized with urethane (175 mg./100 g. of body weight) injected intramuscularly. After 45 to 60 min. the rat was tied to the operating table. A metal cannula was inserted into the trachea. Both vagi and associated sympathetic trunks were cut and 1 mg. of atropine sulphate was injected subcutaneously. The femoral vein close to the inguinal ligament was cannulated with a hypodermic needle mounted in a polythene tube and connected with a burette containing saline. Heparin (50 to 100 units/100 g. of body weight) was injected through the venous cannula. The carotid artery was cannulated with a hypodermic needle mounted in a polythene tube and connected with a mercury manometer of about 2 to 3 mm. internal diameter by a column of normal saline. All solutions were injected through the venous cannula by means of a 1 ml. tuberculin syringe and were washed in with 0.25 ml. of normal saline.

In a few experiments the spinal-rat preparation was used. The animal was anaesthetized, atropinized, its trachea cannulated and both vagi cut, as described above. Clips were put on both carotid arteries temporarily. The spinal cord was cut close to the second

cervical vertebra and artificial respiration was started. The brain was destroyed by a probe thrust through the foramen magnum. A cannula was put into the carotid artery for recording the blood pressure.

Drugs Used.—Urethane (Riedel, S.A.), 17.5 g./100 ml. solution in distilled water. Heparin (Vitrum), 5% solution (5,000 units/ml.). Witte's peptone (Friedr. Witte, Rostock), 10 and 20% fresh solutions in distilled water. These were acidified (*p*H 5), shaken with permutit (1 g. of permutit/g. of peptone), filtered and neutralized prior to use. This treatment freed the solution of contaminating histamine. For most of the experiments Witte's peptone was used although two other peptone preparations have been employed (Pfanstiehl peptone and Bacteriologic Peptone, Park, Davis). The weights of noradrenaline hydrochloride (Delta Chemical Works), (−)-adrenaline bitartrate (Winthrop-Stearns), nicotine (Schering-Kahlbaum) and 5-hydroxytryptamine creatinine sulphate (Sandoz) are those of the base. The quantities of dibenamine hydrochloride (Smith, Kline, and French), dihydroergotamine methane-sulphonate (Sandoz), hexamethonium bromide (Bistrium, Squibb), the butanolamide of lysergic acid (Methergin, Sandoz), atropine sulphate (Poulenc Freres) and dimethylphenylpiperazinium (DMPP) (Parke, Davis) used are expressed as weights of the salts. Pituitrin (Parke, Davis) 5 units/0.5 ml.

RESULTS

The Action of Peptone on the Arterial Blood Pressure.—When peptone in a dose of 10 to 20 mg. (0.05 to 0.1 ml. of a 20% solution)/100 g. of body weight was injected intravenously in atropinized rats usually the blood pressure rose steeply within a few seconds. The first four or five injections caused a definite pressor response, but, on repetition, the same dose or even greater doses of peptone at 5 to 7 min. intervals elicited little or no response at all (tachyphylaxis). Spontaneous recovery of the capacity to produce a new rise was not observed even when the animal was left resting for 3 hr. before testing again. For the purpose of comparing the pressor effect of peptone, a noradrenaline standard solution was used. In view of the gradual desensitization which developed after repeated injections of peptone, it was not possible to bracket the response of the noradrenaline and that of peptone, but a fairly satisfactory comparison between the pressor response of peptone and noradrenaline could be obtained (Fig. 1).

The rise in blood pressure in a group of 30 rats was comparable to that produced by 1 to 10 μg. of noradrenaline. The magnitude of this increase was apparently not dependent upon the initial blood pressure of the animal. In 3 out of 33 rats, however, the injection of peptone failed to produce the pressor effect. All the 3 nonreactive animals showed typical responses to the injection of noradrenaline.

The Rôle of the Adrenal Glands.—Earlier work by Dale and Laidlaw (1912) and by Burn and Dale (1926) in cats indicated that the adrenal glands might be concerned in the pressor responses to pilocarpine and histamine. This led us to investigate the effect of adrenalectomy on the rise in blood pressure following the injection of peptone in the rat. In 8 rats an incision was made in the dorsal region and both adrenal glands excised; 15 to 20 min. later the arterial blood pressure was recorded as described above. A test dose of peptone was injected and the magnitude of

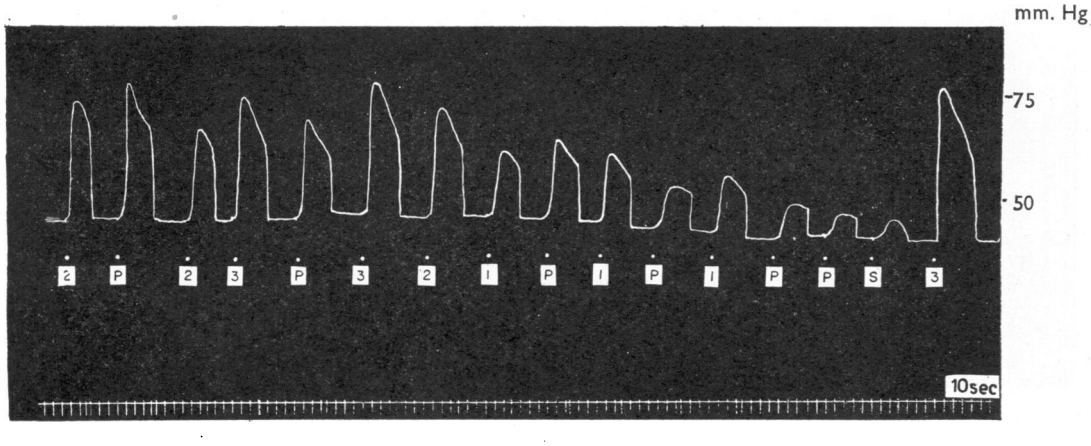

FIG. 1.—Arterial blood pressure of rat (245 g.). Urethane anaesthesia. Vagi cut and atropine given. 1, 2, and 3 = intravenous injections of 1, 2, and 3 μg. noradrenaline. P = 0.25 ml. (0.1 ml./100 g.) 20% solution of Witte's peptone. S = 0.25 ml. saline. The injections were made at 5 to 7 min. intervals.

the pressor response compared with that of noradrenaline. The rise in blood pressure in a group of 8 rats was comparable with that produced by the injection of 2 to 8 μg. of noradrenaline. In 3 rats a pressor response to a test dose of peptone was obtained. Both adrenal glands were then removed and 10 to 15 min. later the test dose of peptone was repeated. Fig. 2 shows the results obtained in one such experiment. Adrenalectomy apparently did not abolish or even reduce the magnitude of the pressor response produced by peptone which cannot therefore be due to the release of sympathin from the adrenal glands.

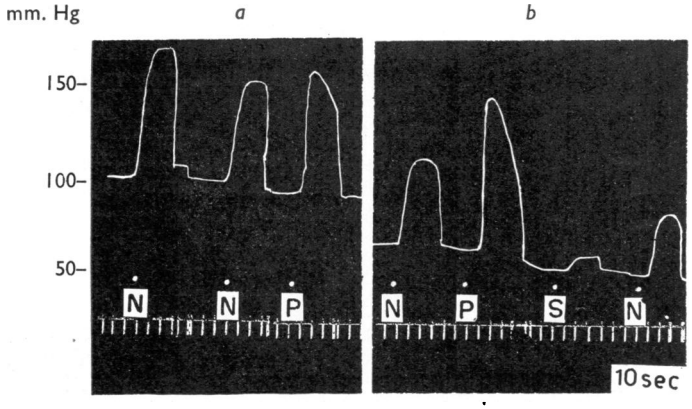

FIG. 2.—Arterial blood pressure of rat (300 g.). Urethane anaesthesia. Vagi cut and atropine given. N=intravenous injections of 2 μg. noradrenaline. P=0.3 ml. (0.1 ml./100 g.) 20% solution Witte's peptone. S=0.3 ml. saline. A period of 15 min. elapsed between (*a*) and (*b*) during which time both adrenals were removed. Before and after adrenalectomy the injections were made at 5 min. intervals.

Drugs Antagonizing the Peptone Pressor Effect.—It is known from the work of Bhattacharya and Lewis (1956) that histamine liberators (48/80, morphine and propamidine) release not only histamine but 5-hydroxytryptamine as well from the perfused hindquarter of rats. If this was the case with peptone, the liberation of 5-hydroxytryptamine might partially or completely account for the pressor effect following the injection of peptone in the rat. In 3 experiments in which the pressor effect of 5-hydroxytryptamine was completely reversed when the animals were treated previously with lysergic acid butanolamide tartrate, a potent inhibitor of 5-hydroxytyptamine, the pressor response to peptone still remained unchanged. Fig. 3 shows the results obtained in one of these experiments.

On the other hand, the pressor effect of peptone was antagonized by anti-adrenaline drugs such as ergotamine, dihydroergotamine, tolazoline, and dibenamine. The results obtained from 12 rats treated with one or other of these blocking agents showed that all of them were effective in reducing or blocking completely the pressor response to peptone. Fig. 4 shows the inhibition of the pressor response to peptone and noradrenaline by dihydroergotamine, under conditions in which

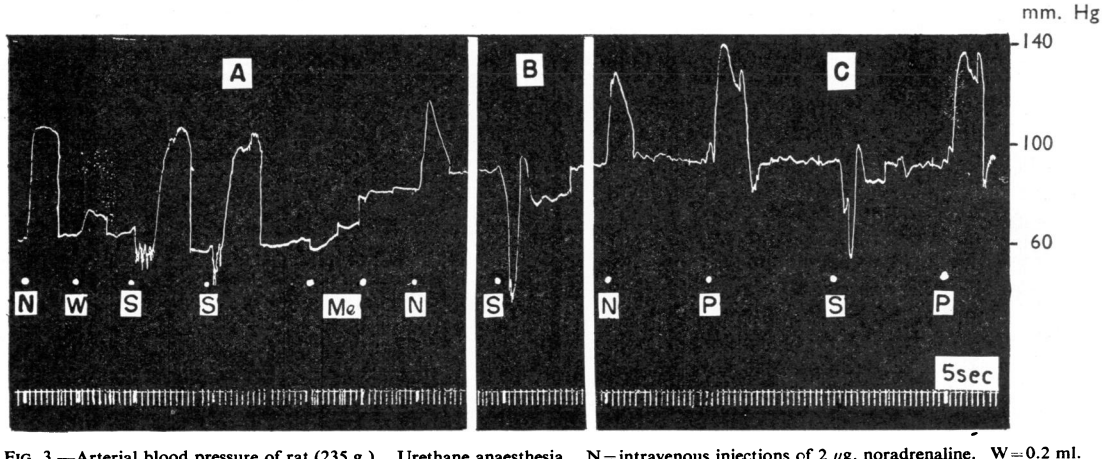

FIG. 3.—Arterial blood pressure of rat (235 g.). Urethane anaesthesia. N=intravenous injections of 2 μg. noradrenaline. W=0.2 ml. saline. S=40 μg. 5-hydroxytryptamine. Me=200 μg. (+)-lysergic acid butanolamide (Methergin) on four repeated injections of 50 μg. each at 2 min. intervals between the two points marked in the recording. P=0.12 ml. (0.05 ml./100 g.) 10% solution of peptone (Parke, Davis). A period of 10 min. elapsed between (A) and (B) and 8 min. between (B) and (C). The animal showed desensitization after the fifth injection of peptone. There was an interval of 5 min. between the injections.

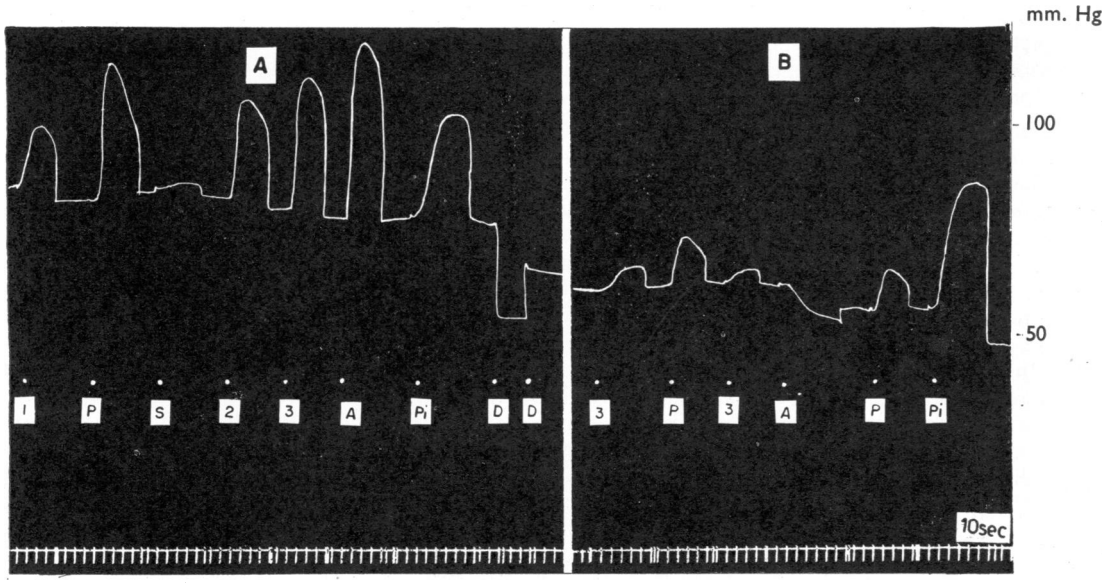

FIG. 4.—Arterial blood pressure of rat (235 g.). Urethane anaesthesia. Vagi cut and atropine given. 1, 2, and 3=intravenous injections of 1, 2, and 3 μg. noradrenaline. P=0.24 ml. (0.1 ml./100 g.) 20% solution Witte's peptone. S=0.25 ml. saline. A=3 μg. adrenaline. Pi=0.015 unit Pituitrin. D=500 μg. dihydroergotamine. A period of 3 min. elapsed between the two injections of dihydroergotamine and of 8 min. between (A) and (B). The injections were made at 5 min. intervals.

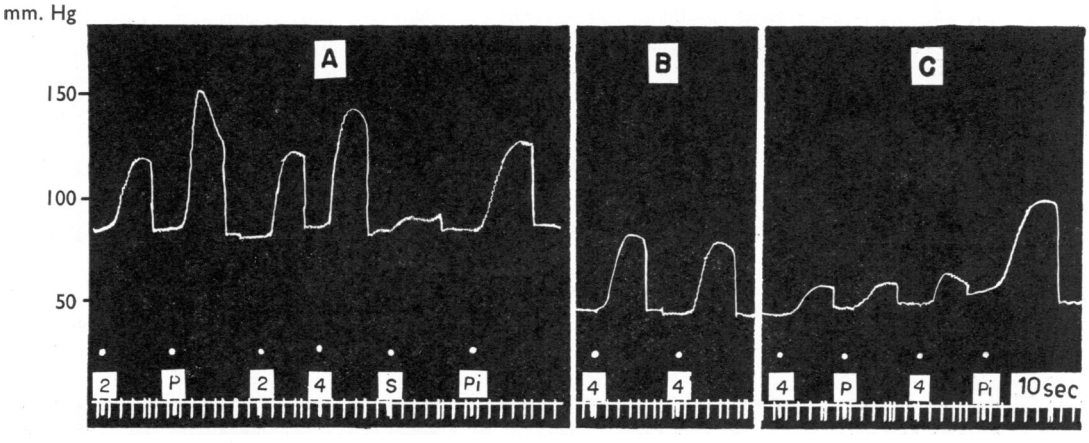

FIG. 5.—Arterial blood pressure of a spinal rat (330 g.). Urethane anaesthesia. Vagi cut and atropine given. 2 and 4=intravenous injections of 2 and 4 μg. noradrenaline. P=0,33 ml. (0.1 ml./100 g.) 20% solution Witte's peptone. S=0.4 ml. saline. Pi=0.01 unit Pituitrin. 5 mg. dibenamine was injected intravenously between (A) and (B) and between (B) and (C). A period of 27 and 40 min. elapsed between (A) and (B) and (B) and (C), respectively. Before and after dibenamine the injections were made at 5 min. intervals.

the pressor response to adrenaline was reversed. From the results of these experiments, it may be suggested that the pressor response to peptone is probably due to the release into circulation of noradrenaline or a noradrenaline-like substance.

Fig. 5 shows the complete blockade of peptone pressor effect by dibenamine in a spinal rat. The purpose of the experiment was to eliminate any influence of the higher nervous centres on the pressor response to peptone.

Nicotine and Hexamethonium.—After a series of intravenous injections of increasing doses of nicotine (50 μg. to 2 mg.), the last of which failed to cause a pressor response, the injection of peptone did not elicit a rise in blood pressure (5 experiments). Fig. 6 shows the results obtained in one such experiment.

In spite of the fact that, in a few experiments, hexamethonium potentiated the pressor response to exogenous noradrenaline it appeared to abolish

completely the pressor response to peptone (8 experiments). Partial recovery of the rise was observed 60 min. later, as shown in Fig. 7.

In order to test the effectiveness of ganglionic blockade after paralysing doses of nicotine and hexamethonium, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), a potent ganglion-stimulating drug studied by Chen, Portman, and Wickel (1951), was used. As both the blockade and recovery of the pressor effect of peptone and DMPP after nicotine and hexamethonium follow a parallel course as shown in Figs. 6 and 7, it is suggested that the effect of peptone in raising the blood pressure is due to stimulation of sympathetic ganglia. The gradual return of reactivity to peptone and ganglion-stimulating agent (DMPP) after the blockade induced by hexamethonium can easily be understood on the basis of the gradual recovery of the sensitivity of the ganglia as the inhibition dies out. The blockade produced by nicotine, on the other hand, remained for the rest of the experiment (90 min.).

Effect of Intravenous Injections of 48/80 and Histamine on the Blood Pressure.—It has been shown recently that histamine stimulates the superior cervical ganglion of the cat (Trendelenburg, 1954, 1955). As it was possible that the histamine released by peptone might cause the stimulation of the sympathetic ganglia, we decided to ascertain whether histamine set free from the tissue by the injection of 48/80 or injection of exogenous histamine would be able to duplicate the rise in blood pressure caused by peptone.

In 6 experiments 48/80 was injected intravenously in doses ranging from 50 μg. to 2 mg. and histamine in doses ranging from 20 to 500 μg./100 g. of body weight (10 experiments). Neither 48/80 nor histamine had any pressor effect, but

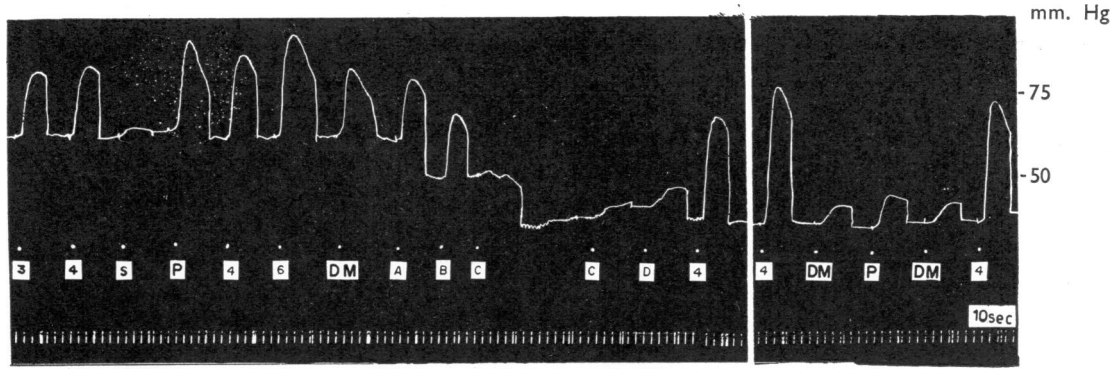

FIG. 6.—Arterial blood pressure of rat (316 g.). Urethane anaesthesia. Vagi cut and atropine given. Artificial respiration. 3, 4, and 6=intravenous injections of 3, 4, and 6 μg. noradrenaline. S=0.3 ml. saline. P=0.3 ml. (0.1 ml./100 g.) 20% solution Witte's peptone. DM=50 μg. 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). Intravenous injections of increasing doses of nicotine 50 μg. (A), 100 μg. (B), 250 μg. (C) and 1 mg. (D). A second series of nicotine injections (10 mg. in all) was given between the two tracings. The injections were made at 5 to 7 min. intervals.

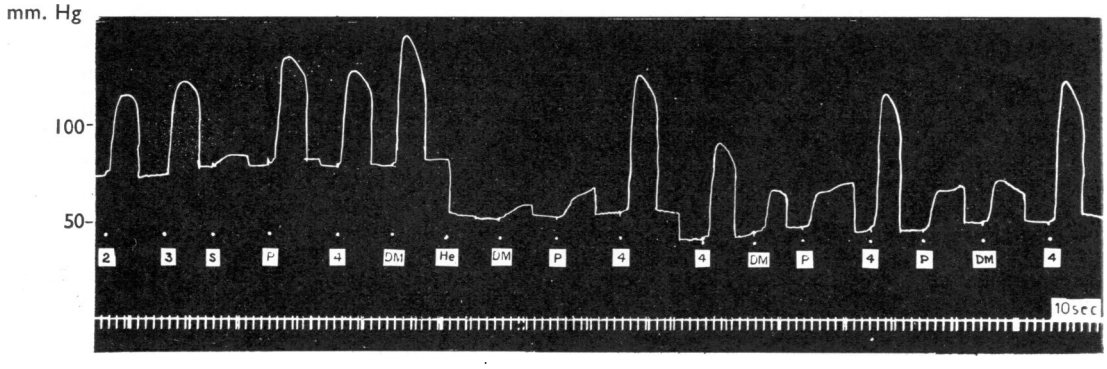

FIG. 7.—Arterial blood pressure of rat (364 g.). Urethane anaesthesia. Vagi cut and atropine given. Artificial respiration. 2, 3, and 4=intravenous injections of 2, 3, and 4 μg. noradrenaline. S=0.4 ml. saline. P=0.36 ml. (0.1 ml./100 g.) 20% solution Witte's peptone. DM=50 μg. DMPP. He=10 mg. hexamethonium. The injections were made at 5 to 7 min. intervals.

all 16 rats showed fall in the blood pressure in both cases. However, the animals showed typical responses to the injection of noradrenaline.

Discussion

The pressor effect of peptone is apparently a normal component of the action of this substance on the blood pressure of the rat. Since the pressor response was not abolished or diminished by adrenalectomy, the blood pressure increase could not have been caused by release of adrenaline or noradrenaline from the adrenal glands. Croxatto and Croxatto (1942) demonstrated that digestion of hypertensinogen (plasma globulin) and other proteins with pepsin leads to the formation of a pressor substance having pharmacological properties very similar if not identical with those of hypertensin (angiotonin). This substance was called pepsitensin. As it was possible that the presence of pepsitensin in peptone could be involved in the mechanism of the rise of blood pressure it is pertinent to emphasize the important pharmacological differences between the pressor response to pepsitensin and that obtained by the intravenous injection of peptone in the rat. According to Alonso, Croxatto and Croxatto (1943) repetition of intravenous injections of pepsitensin did not cause desensitization of the animal (tachyphylaxis), nor was the pressor response to pepsitensin influenced by adrenergic blocking drugs such as 2-piperidinomethylbenzo-1:4-dioxan (933F). On the other hand, desensitization could be observed after repeated injections of peptone and its pressor effect was abolished by adrenergic blocking drugs. In addition, the pressor response to peptone is blocked by paralysing doses of nicotine and hexamethonium. Although no reference to the action of ganglionic blocking agents on the response to pepsitensin could be found, Page and Taylor (1947) reported potentiation of the pressor response by hypertensin (a polypeptide considered homologous if not identical with pepsitensin) by tetraethylammonium. Finally, the peptone solution used throughout these experiments was previously treated with permutit in order to remove trace of histamine, and permutit also adsorbs pepsitensin from aqueous solutions (quoted by Paiva, 1954). Therefore, there is no indication that the pressor effect of peptone is due to the possible presence of pepsitensin in the peptone. The pressor effect also could have been caused by the release of 5-hydroxytryptamine, for it has been shown by Bhattacharya and Lewis (1956) that a series of histamine liberators is able to release 5-hydroxytryptamine from rat tissue. The pressor action of 5-hydroxytryptamine, however, was completely reversed by the butanolamide of lysergic acid (Methergin) under conditions in which the pressor effect of peptone remained unchanged.

Since it was not possible to duplicate the pressor response to peptone by either 48/80 or histamine, the hypothesis put forward that peptone releases sympathin through the histamine set free from the tissues can be ruled out.

The group of drugs which antagonizes or blocks the peptone-induced pressor response both in intact and in spinal rats suggests that the pressor effect may be due to a direct action of peptone on sympathetic ganglia. The antagonistic effect of the adrenergic blocking agents (dibenamine, ergotamine, dihydroergotamine and tolazoline) as that of ganglionic blocking drugs (nicotine and hexamethonium) would thus find an adequate explanation.

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References

Alonso, O., Croxatto, R., and Croxatto, H. (1943). *Proc. Soc. exp. Biol., N.Y.*, **52**, 61.

Beraldo, W. T., and Mota, I. (1953). *Ciencia e Cultura*, **5**, 218.

Bhattacharya, B. K., and Lewis, G. P. (1956). *Brit. J. Pharmacol.*, **11**, 202.

Burn, J. H., and Dale, H. H. (1926). *J. Physiol.*, **61**, 185.

Chen, G., Portman, R., and Wickel, A. (1951). *J. Pharmacol.*, **103**, 330.

Croxatto, H., and Croxatto, R. (1942). *Science*, **95**, 101.

Dale, H. H., and Laidlaw, P. P. (1912). *J. Physiol.*, **45**, 1.

Dragstedt, C. A. (1937). *J. Pharmacol.*, **59**, 429.

Feldberg, W., and O'Connor, W. J. (1937). *J. Physiol.*, **90**, 288.

Page, I. H., and Taylor, R. D. (1947). *Science*, **105**, 622.

Paiva, A. C. M. (1954). *Estudo comparativo entre a Pepsitensina e a Hipertensina.* Thesis, Escola Paulista de Medicina, S. Paulo: Tip. Rossolillo.

Rocha e Silva, M., Teixeira, R. M., and Andrade, S. O. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 376.

Root, M. (1951). *J. Pharmacol.*, **101**, 125.

Slater, I. H., and Dresel, P. E. (1952). Ibid., **105**, 101.

Trendelenburg, U. (1954). *Brit. J. Pharmacol.*, **9**, 481.

—— (1955). *J. Physiol.*, **129**, 337.