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THE PHARMACOLOGICAL ACTIONS OF CAPSAICIN AND ANALOGUES

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Capsaicin, a pungent principle present in various species of *Capsicum*, is a decylenic acid amide [I,R = $-(CH_2)_4CH:CH.CH(CH_3)_2$] of vanillylamine.

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On varying the acid portion of the molecule different degrees of pungency of the analogues have been observed (Nelson, 1919; Ott and Zimmermann, 1921; Jones and Pyman, 1925; Ford-Moore and Philips, 1934). Other pharmacological effects of various species of *Capsicum* have been reported. Thus Högyes (1878) first observed that the burning sensation produced by an alcoholic extract of *Capsicum annuum* dropped upon the skin was accompanied by hyperaemia. Lille and Ramirez (1935) found that intravenous injection of an extract of *Capsicum* into the dog produced a fall in blood pressure accompanied by a variable effect on the respiration, an increase in salivary secretion and a relatively small increase in gastric secretion. They also observed that capsaicin extracts increased the activity of the isolated rabbit intestine and guinea-pig uterus, although Nast (1923) had previously found that there was a relaxation of the isolated rabbit intestine. Capsaicin also lowers the rectal temperature of the mouse when given subcutaneously (Issekutz, Lichtneckert, and Nagy, 1950)—an effect interpreted as being due to excitation of heat receptors.

Heubner (1925) found that undecylenacidic—allinyl—amide, an acrid substance related to capsaicin, caused sweating of the forehead of man when smeared upon the mucous membrane of the mouth. In a study of gustatory sweating in healthy people Lee (1954) used the fresh fruits of *Capsicum frutescens, L.*, or *C. minimum, Roxb.* He produced evidence that the reflex was initiated by pain fibres and showed that reflex sweating could not be induced by application of *Capsicum* to the mucosa of the oesophagus or stomach. He found, however, that *Capsicum* produced profuse salivation when placed in the mouth. The oleoresin of *Capsicum* has long been recognized in the *B.P.* and *U.S.P.* as a powerful gastro-intestinal stimulant and as a rubefacient.

The effects obtained with whole fruits or crude extracts of *Capsicum* can no doubt be attributed in part to capsaicin. Since some of the effects described could be due to substances other than capsaicin, the pharmacological actions of pure capsaicin and some of its analogues were investigated on animals with particular reference to the circulation and respiration.

The compounds studied were capsaicin, vanillyl-*n*-decoylamide [I,R = $-(CH_2)_8CH_3$], vanillyl acetamide [I,R = $-CH_3$] and vanillylamine.

METHODS

Cats under chloralose anaesthesia were used; the trachea was cannulated and respiration was recorded by measuring the tidal air volume with a closed circuit spirometer filled with oxygen. The blood pressure was recorded either from the femoral or from the carotid artery. Intravenous injections were made direct into the femoral vein, but injections into all other blood vessels were made through cannulae. Injections into the carotid sinus region were made through the lingual artery after clamping the external carotid artery at a point near the origin of the lingual artery, but without tying the occipital and ascending pharyngeal arteries. Injections into the superior mesenteric artery were made through a branch of that artery. Injections into the femoral artery were made through a branch supplying the muscles. Injections into the saphenous artery were made through a cannula inserted into the popliteal artery so that the tip of the cannula was just below the origin of the saphenous artery, and all other branches of the femoral artery were tied off. Injections into the sartorius and quadriceps muscles were made through the femoral artery with the tip of the cannula lying below and pointing towards the origin of the lateral circumflex femoral artery.

Injections into the right auricle were made through a silicone-treated glass catheter which was passed down the left external jugular vein until its tip had entered the auricle. For injection into the left ventricle a similar catheter was passed down the left common carotid artery until its tip had entered the left ventricle. The catheters were filled with a solution of the substance to be injected and 0.2 ml. was injected into the heart each time. At the end of each experiment the chest was opened to determine whether the tips of the catheters were in the chambers of the heart.

Cooling of the vagi was carried out essentially according to the method of Partridge (1939). Dry air, previously chilled by passing through coils covered with frozen CO_2, was circulated through hollow tin thermodes on which the vagi lay. By varying the rate of circulation of cold air the vagi could be cooled to the requisite temperature. The temperature of the surface of the thermodes was measured by a thermocouple.

Gastric juice was collected from a catheter inserted into the stomach through an opening in the duodenum, the cat lying on its right side (Wood, 1948).

The vascular effects of capsaicin were studied on the blood vessels of the isolated rabbit's ear which was perfused with Locke solution aerated with oxygen.

Isolated guinea-pig ileum was suspended in 20 ml. Tyrode solution aerated with 95% O_2 and 5% CO_2. Isolated rat uterus was suspended in 20 ml. of a solution deficient in Ca^{++}, prepared according to the formula of Gaddum, Peart, and Vogt (1949) and aerated with O_2.

Capsaicin was isolated from the fresh fruits of *Capsicum frutescens, L.*, or *C. minimum, Roxb.* Its isolation and the preparation of its analogues are described in the chemical section at the end of this paper.

Vanillylamine was used as the hydrochloride. Capsaicin and vanillyl-*n*-decoylamide were not readily soluble in water. They were dissolved in 0.1 N-NaOH and the solution was gradually acidified with 0.1 N-HCl until the *p*H was about 7–8. Thus solutions containing 100 μg./ml. capsaicin and 50 μg./ml. vanillyl decoylamide were obtained.

RESULTS

Intravenous Injection

Injections of 20–40 μg. capsaicin into the femoral vein produced apnoea in the expiratory phase and usually a biphasic effect on the blood pressure, the depressor phase occurring first and being sometimes the only response (Fig. 1). When the vagi were sectioned the apnoeic response and the depressor effect were no longer obtained, but there was a strong pressor effect accompanied by an increase in the respiratory volume. In two out of six cats, however, the depressor effect and decrease in respiratory volume were still obtained but were considerably reduced.

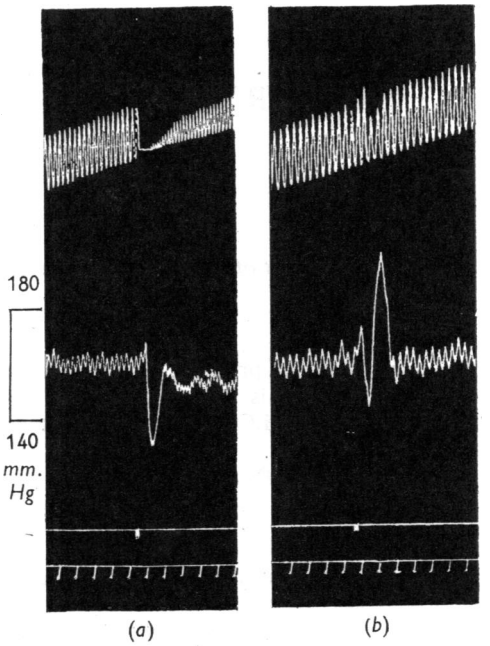


FIG. 1.—Cat, ♂, 3 kg., chloralose. Records: respiration, blood pressure and time (10 sec.). Effect of capsaicin (20 μg.) i.v. Between (*a*) and (*b*) the vagi were cut in the neck.

Effect of Cooling the Vagi.—On cooling the vagi with thermodes at 9–10° C. the apnoeic effect of capsaicin was completely abolished in some experiments, but in others it was only reduced. This residual effect on the respiration, however, could be completely abolished by further cooling the vagi to 1.5–3.5° C. (Fig. 2). The depressor effect of capsaicin was more readily abolished by cooling the vagi, the effect disappearing at 8–10° C.

Injection into the Heart

Capsaicin (25 μg.) injected into the right auricle produced apnoea after a latent period of 1.5 sec., but injection into the left ventricle produced hyperpnoea instead. After the vagi were sectioned injection into the right auricle no longer produced apnoea, but injection into the left ventricle continued to produce hyperpnoea (Fig. 3). In the intact animal, a marked depressor effect was obtained almost instantaneously on injection of 25 μg. of capsaicin into either the left ventricle or the right auricle. On sectioning the vagi a pressor effect was obtained in each case.

Injection into the Carotid Sinus Region

When 10 μg. capsaicin was injected into the carotid sinus there was apnoea and a fall of blood pressure (Fig. 4). On sectioning the sinus nerve or the IXth nerve close to its entrance into the

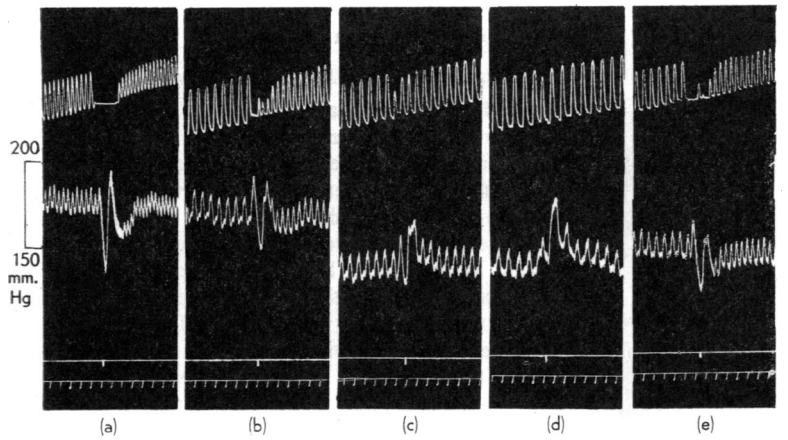
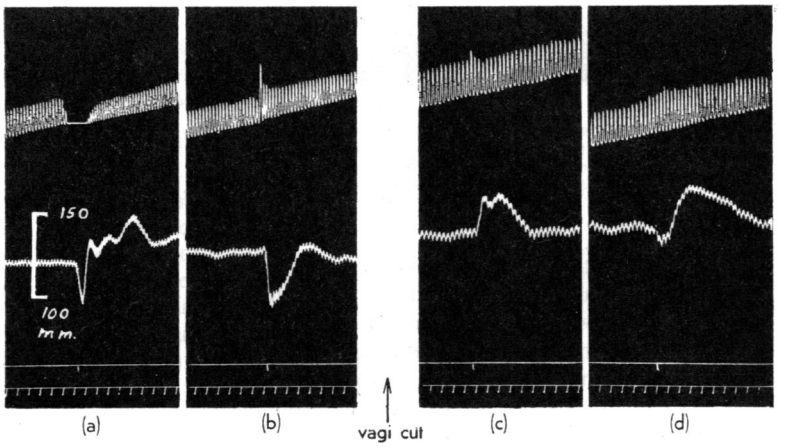


FIG. 2.—Cat, ♂, 3.1 kg., chloralose. Records: respiration, blood pressure and time (10 sec.). The effect of capsaicin (20 μg.) i.v.: (*a*) before cooling cervical vagi, (*b*) on cooling vagi to 8.7° C., (*c*) to 4.1° C., (*d*) to 2.7° C., and (*e*) after recovery from cooling.

FIG. 3.—Cat, ♀, 3.25 kg., chloralose. Records: respiration, blood pressure and time (10 sec.). Capsaicin (25 μg.) was injected into the right auricle at (*a*) and (*c*) and into the left ventricle at (*b*) and (*d*). Between (*b*) and (*c*) the vagi were sectioned.



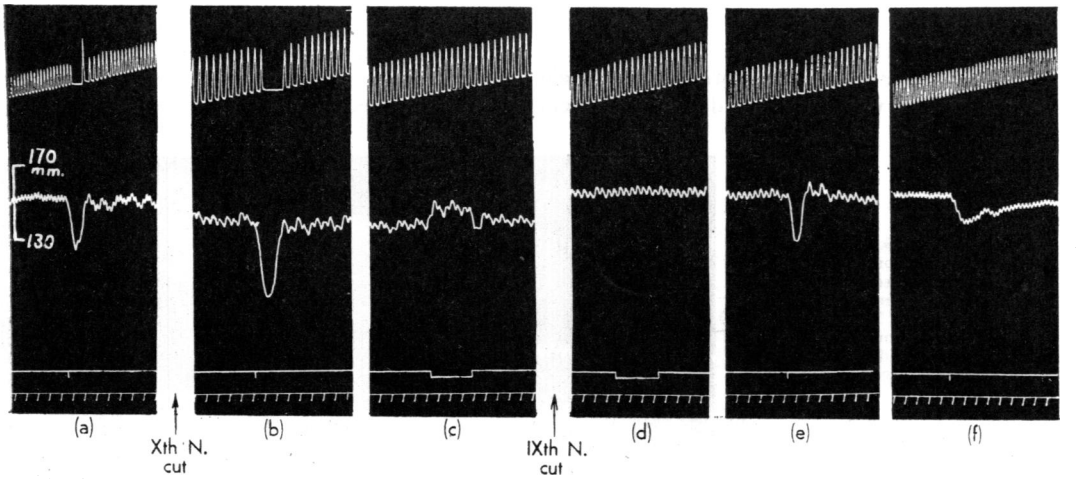


FIG. 4.—Cat, ♂, 2.75 kg., chloralose. Records: respiration, blood pressure and time (10 sec.). The effect of capsaicin (10 μg.) injected into the right carotid sinus at signal marking in (*a*), (*b*), (*e*), and (*f*). The vagi were cut below the sinuses between (*a*) and (*b*). The IXth nerve was cut between (*c*) and (*d*). In (*c*) and (*d*) the right common carotid artery was clamped for the period indicated by the signal marker. In (*f*) the carotid region was covered throughout with a solution of cocaine.

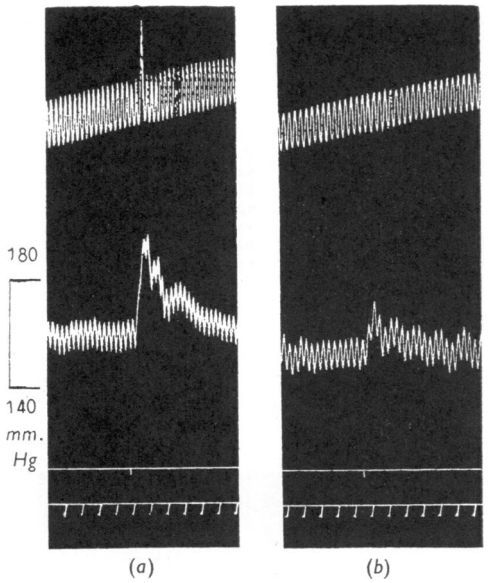


FIG. 5.—Cat, ♀, 2.5 kg., chloralose. Records: respiration, blood pressure and time (10 sec.). Effect of capsaicin (10 μg.) injected into the superior mesenteric artery. The superior and inferior mesenteric nerve plexuses were infiltrated with procaine between (*a*) and (*b*).

skull the respiratory apnoea was considerably reduced in three cats and abolished in one, while the depressor effect was reduced in all the cats but not abolished. When the carotid sinus region and the exposed common carotid artery in the neck were covered with a solution of cocaine, injection of capsaicin into the carotid sinus no longer produced any effect on the respiration, although there was a slow and slight fall in blood pressure which gradually returned to its original level (cf. Fig. 4e and f). This depressor effect, therefore, had its origin elsewhere and it was probably due to capsaicin being carried away through untied vessels, such as the occipital and ascending pharyngeal arteries.

Injection into the Splanchnic Circulation

When 10 μg. capsaicin was injected into the superior mesenteric artery, there was a rise in the systemic blood pressure accompanied in two experiments by hyperventilation, and in two others by a decrease in the inspiratory volume (Fig. 5). These responses were not affected by cutting the vagi in the neck, but they were abolished either by extirpating the superior and inferior mesenteric plexuses with their ganglia or by infiltrating these nerve plexuses with procaine. Usually, after abolition of these nervous reflexes, a further injection of capsaicin into the superior mesenteric artery produced a small rise in the systemic blood pressure, although it had no effect on the respiration. This residual effect on the blood pressure is due to mechanical occlusion of the splanchnic vessels brought about by contraction of the intestinal muscular wall in response to capsaicin.

Injection into the Leg

When capsaicin (10–20 μg.) was injected into the femoral artery it caused hyperpnoea, sometimes preceded by a brief period of apnoea. The effect on the blood pressure was variable, being either pressor or depressor, and sometimes altogether absent. Twitching of the leg muscles was occasionally observed. These effects were not abolished or reduced on cutting the saphenous and medial cutaneous nerves. In an experiment in which capsaicin (20 μg.) was injected into the saphenous artery pressor and hyperpnoeic effects were observed. These effects were not reduced on skinning the leg, but were completely abolished by sectioning the sciatic and femoral nerves (Fig. 6). These results suggested that capsaicin was acting on nervous receptors in the muscles. On injecting

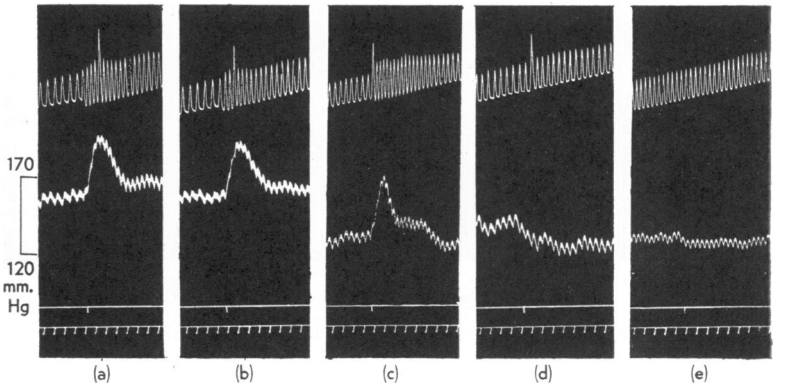


FIG. 6.—Cat, ♂, 3.3 kg., chloralose. Records: respiration, blood pressure and time (10 sec.). Effect of capsaicin (20 μg.) injected into the saphenous artery. The saphenous and medial cutaneous nerves were cut between (*a*) and (*b*). The leg was skinned between (*b*) and (*c*). Between (*c*) and (*d*) the sciatic nerve was cut and between (*d*) and (*e*) the femoral nerve was cut.

capsaicin (10–20 μg.) into the blood supply of the sartorius and quadriceps there was frequently an increased respiratory response accompanied by a slight pressor or depressor effect; all these effects were abolished by sectioning the femoral nerve.

Other Observations on the Pharmacological Action of Capsaicin

Perfused Rabbit Ear.—Capsaicin in doses as large as 100 μg. did not have any effect on the perfused rabbit ear vessels, although 0.3 μg. adrenaline was sufficient to constrict the blood vessels. In one experiment 10 μg. capsaicin caused a vasodilatation in the ear vessels which could be abolished by a previous injection of procaine (50 μg.) into the perfused ear, but this result could not be repeated.

Isolated Guinea-pig Intestine.—Capsaicin contracted the guinea-pig ileum in concentrations of 0.1 μg./ml. The preparation, however, rapidly became tachyphylactic to further doses of capsaicin, although it still responded to histamine or acetylcholine.

Isolated Rat Uterus.—Concentrations of 1 μg./ml. capsaicin did not contract or relax the rat uterus, although 0.05 μg./ml. carbachol caused contraction and 0.05 μg./ml. adrenaline complete inhibition.

Gastric Secretion.—Capsaicin did not affect HCl secretion in the cat either when injected in doses of 0.5 mg. subcutaneously or when 1 mg. was left in the stomach for 15 min., although a test dose of histamine (0.5 mg.) increased acid secretion when injected subcutaneously. When capsaicin was left in the stomach, there was, however, a small secretion of mucus.

Pharmacological Activity of Analogues of Capsaicin

Vanillylamine.—Vanillylamine hydrochloride itself did not have any action on the isolated guinea-pig ileum or rat uterus in concentrations of 4 μg./ml. In the cat 5 mg. of the amine injected intravenously had no effect on the respiration, but there was a slight and gradual rise in the blood pressure which occurred about 10–15 sec. after injection quite unlike the sharp rise in blood pressure obtained with capsaicin. The blood pressure gradually returned to its normal value.

Vanillyl Acetamide.—This acetyl derivative of vanillylamine, like vanillylamine itself, did not contract or inhibit the isolated rat uterus (50 μg./ml.). The guinea-pig ileum was stimulated by 0.2 μg./ml. vanillyl acetamide, but quickly became tachyphylactic to it. Intravenous injection of 2 mg. into the cat had no effect either on the respiration or on the blood pressure, although a marked apnoea and a strong rise in blood pressure were observed with 20 μg. of vanillyl-*n*-decoylamide.

Vanillyl-n-decoylamide.—Vanillyl-*n*-decoylamide produced very much the same effects as capsaicin. However, it had a greater effect on respiration than on the blood pressure, and apnoea could be obtained with small doses of the decoylamide (10 μg.), although a depressor effect on the blood pressure was not always obtained. More often this substance produced a rise in the systemic blood pressure. Vagotomy, or cooling the vagi to 9–10° C., abolished the apnoeic response but not the pressor effect.

The decoylamide produced apnoea when injected in doses of 10 μg. into the right auricle, and this effect was abolished by vagotomy; but injection into the left ventricle increased the depth of respiration and was not affected by vagotomy.

Pupillary dilatation was frequent with intravenous injections of the *n*-decoylamide, even when the superior cervical ganglion was extirpated. The *n*-decoylamide and capsaicin had similar effects on the isolated intestine and uterus.

Capsaicin and vanillyl-*n*-decoylamide produced tachyphylaxis when injected successively at short intervals, but the blood-pressure responses became tachyphylactic more readily than the respiratory responses. Thus, although the effects on the blood pressure became irregular, apnoea could still be readily elicited.

Discussion

Enough evidence has been collected to show that capsaicin stimulates sensory nerve endings to bring about reflex changes in the systemic blood pressure and respiration which vary with the site of action of the substance. The afferent pathways for these reflex effects include the vagi, the sinus nerves, the splanchnic nerves and the nerves to the skeletal muscles. From the discussion which follows it is concluded that capsaicin, besides acting on chemoreceptors, can also stimulate or sensitize stretch receptors in the lungs and baroreceptors in the carotid sinus.

The apnoeic and depressor effects seen when capsaicin was injected into the chambers of the heart resembled closely those obtained with veratridine (Dawes, 1947; Aviado, Pontius and Schmidt, 1949), 5-hydroxytryptamine (Mott and Paintal, 1953; Schneider and Yonkman, 1953) and the amidines (Dawes and Mott, 1950). The fact that small doses of capsaicin produced apnoea only when injected into the right auricle but not

into the left ventricle suggests that the site of action of capsaicin is probably in the pulmonary circulation. If the observation that cooling of the vagi to about 9–10° C. blocks conduction in slowly adapting pulmonary stretch fibres (Whitteridge, 1948) is related to the present finding that the apnoea produced by capsaicin was greatly reduced or abolished by cooling the vagi to a similar temperature, then it must be concluded that capsaicin acts largely by sensitizing the Hering-Breuer inflation reflex. In some experiments, however, there still remained a small effect on the respiration which was not abolished until the vagi were cooled to a temperature between 1.5° and 3.5° C.; in this respect capsaicin had an action which resembled that of the amidines and which may be mediated by unidentified chemoreceptors in the lungs (Dawes, Mott, and Widdicombe, 1951).

The sudden fall in blood pressure when capsaicin was injected intravenously, or into the left ventricle, is reminiscent of a similar effect first observed with veratrine by von Bezold and Hirt (1867). This effect is partly due to an action on nerve endings in the lungs (Richter and Amann, 1940) and to a greater extent on nerve endings in the heart (Dawes, 1947). Although capsaicin was not injected directly into the coronary arteries, it is very likely that the depressor effect was partly due to a coronary chemoreflex, for it was obtained almost instantaneously with small doses of capsaicin injected into the left ventricle, and it was abolished by vagotomy. Criteria for distinguishing chemoreflexes from the heart and lungs have been given by Dawes and Comroe (1954), but it cannot be ascertained from these whether the depressor effect when capsaicin was injected into the right auricle was a pulmonary depressor effect.

The apnoeic and depressor effects produced on injecting capsaicin into the carotid sinus region were no doubt mediated largely by the sinus nerve. The fact that these effects could not be abolished by sectioning the IXth nerve indicates that other nerve fibres, probably branches from the vagus, also serve as afferent pathways. To what extent the carotid body or the carotid sinus pressor receptors are responsible for these effects has not been determined. It is interesting, however, to suggest the possibility that just as pulmonary stretch receptors can be sensitized so also may baroreceptors in the carotid sinus walls, and it may be the sensitization of these baroreceptors that evoked the reflex fall in blood pressure. Whether the apnoea, if it is at all due to the action of capsaicin on the carotid body, can be related to an increase in blood flow through the carotid body has yet to be determined. An increase in blood flow, however, would appear to be unlikely, as capsaicin has not been found to have a strong vasodilator effect and as there was a fall in blood pressure when capsaicin was injected into the carotid sinus region.

Injection of capsaicin intravenously into an animal whose vagi had been cut at the neck or into the aorta almost always produced a rise in blood pressure and an increase in respiration. These effects are reflex in origin and can be partly attributed to the action of capsaicin in the abdominal viscera and skeletal muscles.

That no reflex effects have been shown to arise from the action of capsaicin on the skin is particularly surprising, as it is well known that it has an irritating action even when spread on the skin. It has not been determined whether the reflex hyperpnoea produced by injecting capsaicin into the muscles was due to stimulation of pain fibres or other nervous receptors. More interesting, however, is the remarkable reflex rise in blood pressure when capsaicin was injected into the splanchnic circulation. Apart from finding that the sympathetic nerve fibres form the afferent pathway, the location of the receptors stimulated has yet to be determined. It would be interesting to find out the physiological role of these receptors in the viscera.

The experiments with the analogues of capsaicin suggest that their pharmacological activity is dependent on the nature of the acyl radical to which vanillylamine is conjugated. Nelson (1919) has shown that there is a relationship between the degree of pungency and the length of the acyl radical. Pharmacological activity may bear a similar relationship to structure: the acetyl derivative of vanillylamine, besides lacking in pungency, had no activity, whereas the acrid decoyl derivatives, with or without an unsaturated bond in the fatty acid chain, showed strong activity.

Capsaicin and its active analogues appear to be potent pharmacological tools for studying the physiological activity of sensory nerve endings.

Chemical Section

The m.p.s. are uncorrected. Microanalyses were carried out by Dr. W. Zimmermann, C.S.I.R.O., Melbourne.

Isolation of Capsaicin.—The method used was a modification of that employed by Tice (1933) and that by Büchi and Hippenmeier (1948). It appears to take a shorter time and may be adapted to the isolation of capsaicin from either the oleoresin or the dried fruit.

Fresh fruit (3 kg.) of *Capsicum frutescens, L.*, or of *C. minimum, Roxb.*, were macerated with acetone (100 g./150 ml. acetone). The acetone was run off the next day and the residue extracted by percolation

3 times more, each time with 2 l. of acetone. The acetone was removed under diminished pressure and the resulting aqueous oily mixture (about 1.8 litres) was shaken with chloroform (500, 300 ml.). The chloroform solution was shaken with 5% NaOH (800 ml.). On the next day the chloroform layer was carefully separated off. Concn. HCl (64 ml.) was added to the alkaline extract to neutralize most of the alkali, and CO_2 was passed into the solution until it was no longer blue to thymol blue. The solution was extracted with ether (400, 300, 200 ml.) and after being dried with anhydrous sodium sulphate (20 g.) the ether was removed. The residue was dissolved in 10 times its volume of warm 2% NaOH and any oil remaining undissolved was removed by light petroleum (b.p. 60–80°). Crude capsaicin was precipitated by the addition of a warm saturated solution of ammonium chloride and extracted with ether. Removal of the ether after drying with anhydrous sodium sulphate left an oily residue from which the first crystals were obtained by molecular distillation at 205–215° at 0.3–0.5 mm. In subsequent isolations the oily residue obtained at the last stage was rubbed with light petroleum and seeded with capsaicin, the container being placed in ice. After the oil became semi-solid the mixture was placed in a refrigerator for 24 to 48 hr. The solid mass was filtered off and extracted repeatedly with hot light petroleum (b.p. 80–100°). Crude capsaicin crystallized out from the extracts on chilling and rubbing with a few crystals of capsaicin. The yield of crude capsaicin (m.p. 55–60°) was 0.8–1.5 g. After three recrystallizations from light petroleum the capsaicin melted at 63–64°. (Found : C, 70.3 ; H, 9.0. $C_{18}H_{28}O_3N$ requires C, 70.6 ; H, 9.2%.)

Other Preparations.—Vanillylamine and its hydrochloride were prepared according to the method of Challis and Clemo (1947), and vanillyl-*n*-decoylamide according to that of Nelson (1919).

Vanillyl acetamide was prepared by suspending vanillylamine (2 g.) in water (8 ml.) and gradually adding acetic anhydride (2 ml.). After the mixture was heated on a steam bath for 5 min. it was cooled and the amide was extracted with chloroform (3 × 5 ml.). Removal of chloroform after drying gave an oil which crystallized after several days. In subsequent preparations crystals were readily obtained from the oil by rubbing with light petroleum and seeding with crude vanillyl acetamide. Thrice recrystallized material from benzene gave colourless crystals, m.p. 98–99°. (Found : C, 61.90 ; H, 6.85 ; N, 6.9. $C_{10}H_{13}O_3N$ requires C, 61.5 ; H, 6.7 ; N, 7.2%.) Nelson (1919) gave m.p. 84–85°.

SUMMARY

1. A modified method for isolating capsaicin from *Capsicum* is described.

2. Intravenous injection of capsaicin produces apnoea which is either abolished or greatly reduced by cooling the vagi to 9–10° C. Further cooling the vagi to 2–3° C. abolishes any residual apnoea. This effect is traced to an action on receptors, probably stretch receptors, in the lungs.

3. Capsaicin causes a fall in blood pressure and heart rate which is abolished by cutting the vagi. This effect is probably a coronary chemoreflex.

4. Capsaicin causes apnoea and a fall in blood pressure when it is injected into the carotid sinus region ; these effects are greatly reduced by sectioning the sinus nerve. It is suggested that the depressor effect is due to sensitization of the carotid sinus baroreceptors.

5. Capsaicin acts on undetermined receptors in the intestines, provoking a reflex rise in blood pressure which can be abolished by extirpating the superior and inferior mesenteric plexuses.

6. Capsaicin acts on undetermined receptors in the skeletal musculature, usually causing hyperpnoea and a variable effect on the systemic blood pressure. These effects are abolished by sectioning the nervous supply to the muscles.

7. Capsaicin has no effect on gastric acid secretion.

8. Capsaicin contracts guinea-pig ileum which, however, readily becomes tachyphylactic to the substance. It does not stimulate or inhibit the isolated rat uterus.

9. Capsaicin has no direct action on the blood vessels of the perfused rabbit ear.

10. Vanillylamine and vanillyl acetamide have little or no activity. Vanillyl-*n*-decoylamide has the same pharmacological activity as capsaicin.

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