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## The inhibition of adrenergic α-receptors and tryptamine receptors by macusine B

SIR,—Macusine B, an alkaloid isolated from *Strychnos toxifera*, blocks α-adrenergic receptors, stimulates β-adrenergic receptors and competitively inhibits tryptamine receptors in the guinea-pig isolated ileum (Leonard, 1965). A quantitative comparison has now been made with other compounds known to inhibit these receptors.

The antagonism of 5-hydroxytryptamine (5-HT) by macusine B was measured on the guinea-pig isolated ileum and the rat isolated uterus preparations. These tissues were suspended in an organ bath of Tyrode and De Jalon solutions respectively, as described previously (Leonard, 1965), and the $pA_2$ and $pA_{10}$ values measured (Schild, 1947). The inhibition of adrenergic receptors was assessed using the guinea-pig isolated vas deferens preparation (Leach, 1956), and on the rabbit aortic strip preparation of Furchgott & Bhadrakom (1953). A modified aorta strip was prepared. A 3 cm length of aorta was first slit longitudinally, placed on a filter paper moistened with Krebs solution and then cut from each side in the horizontal plane so that the cuts alternated but did not extend to the mid-line. By leaving an uncut portion 1 mm wide down the midline and alternating the horizontal cuts by about 1 mm, a robust strip was achieved.

The aorta was then suspended in an organ bath of Krebs solution and attached to a frontal writing lever, backweighted by a load of 0·5 g, giving a × 10 magnification. Aorta prepared in this way gave regular responses to adrenaline ($2–4 \times 10^{-7}$M) for at least 12 hr.

In all experiments the pA values were measured on tissues from at least two animals and the $pA_2$ and $pA_{10}$ values were measured separately on different pieces of tissue from the same animal. The $pA_2$ and $pA_{10}$ values for macusine B on all the tissues were in the range 5·02–6·87 and 4·01–5·77 respectively; there was a slight increase in the value (0·08–0·65 pA units) when the contact time of the antagonist was increased from 2 to 10 min (Table 1). Schild (1957) calculated the $pA_2$–$pA_{10}$ value for a first order competitive antagonist to be 0·95.

TABLE 1. ANTAGONISM OF ADRENALINE AND 5-HT BY MACUSINE B EXPRESSED AS $pA_x$ VALUES

| Tissue | Bath fluid and temperature | Concentration of agonist | Agonist contact time | Antagonist contact time | $pA_2$ | $pA_{10}$ | $pA_2$–$pA_{10}$ |
|---|---|---|---|---|---|---|---|
| Rabbit aortic strip | Krebs; 37° | Adrenaline; $2–4 \times 10^{-7}$M | 3 min | 10 min | 6·674 | 5·771 | 0·903 |
| Guinea-pig vas deferens | Tyrode; 37° | Adrenaline; $4–8 \times 10^{-6}$M | 30 sec<br>,, | 2 min<br>10 min | 5·569<br>5·905 | 4·011<br>4·627 | 0·578<br>0·778 |
| Guinea-pig ileum .. | Tyrode; 37° | 5-HT; $1·8–3·6 \times 10^{-7}$M | 20 sec<br>,, | 2 min<br>10 min | 5·016<br>5·097 | 4·051<br>4·661 | 0·965<br>0·418 |
| Rat uterus .. | De Jalon; 30° | 5-HT; $1·8–3·6 \times 10^{-7}$M | 20 sec<br>,, | 2 min<br>10 min | 6·220<br>6·870 | 5·647<br>5·068 | 0·573<br>0·802 |

As the $pA_2$–$pA_{10}$ values for macusine B are much lower than this for the uterus and vas deferens it seems likely that the alkaloid is acting in a different way on the receptors in these tissues.

Table 2 gives a comparison of the $pA_2$ values for several antagonists of adrenaline and 5-HT. It is evident that macusine B is a weak inhibitor of

TABLE 2. COMPARISON OF THE $pA_2$ VALUES FOR AGONISTS OF ADRENALINE AND 5-HT

| Tissue | Agonist | Antagonist | Contact time | $pA_2$ | Reference |
|---|---|---|---|---|---|
| Rabbit aortic strip .. | Adrenaline | Dihydroergotamine | — | 7·70 | Calculated from data of Furchgott (1955) |
| | | Phentolamine | — | 7·52 | |
| | | Yohimbine | — | 6·70 | |
| | | Macusine B | 10 min | 6·67 | |
| | | Piperoxan | 10 min | 6·28 | Birmingham & Szolcsányi (1965) |
| Guinea-pig vas deferens .. | Noradrenaline | Dihydroergotamine | 30 min | 8·25 | Birmingham & Szolcsányi (1965) |
| | ,, | Piperoxan | 5 min | 6·47 | Calculated from data of Leach (1956) |
| | Adrenaline | Macusine B | 10 min | 6·07 | |
| Rat uterus .. | 5-HT | Lysergic acid diethylamide | 10 min | 8·70 | Gaddum (1953) |
| | | Dibenamine | 10 min | 7·72 | Gaddum, Hameed, Hathaway & Stephens (1955) |
| | | Dihydroergotamine | 10 min | 6·90 | |
| | | Macusine B | 10 min | 6·87 | Gaddum, Hameed Hathaway & Stephens (1955) |
| | | 5-Benzyloxygramine | 10 min | 6·79 | |

adrenergic and tryptamine receptors. On the rabbit aorta and the rat uterus this alkaloid is approximately equipotent with yohimbine and 5-benzyloxygramine respectively.

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## The preparation of *O*-methylporphyroxine and its detection in opium

SIR,—Pfeifer (1965) has recently reported the detection of papaverrubin B (*O*-methylporphyroxine) in opium, but no experimental details were given. We have prepared *O*-methylporphyroxine and found it to differ from that described by Pfeifer & Teige (1962) in m.p. and infrared absorption spectrum. We have also detected *O*-methylporphyroxine in Japanese opium.

Porphyroxine was isolated from Japanese opium (2 kg) essentially by the method of Pfeifer & Teige (1962), although the later stages of their procedure (precipitation of thebaine hydrogen tartrate and column chromatography) were omitted since porphyroxine could be crystallised from methanol without these steps. Its m.p., 234–236°, was undepressed on admixture with a sample isolated from Indian opium by Genest & Farmilo (1963). The mother liquors were recycled through the extraction procedure until no more porphyroxine could be separated by crystallisation. The final filtrate was shown by thin-layer chromatography to contain, in addition to all the common opium alkaloids, porphyroxine and traces of several other compounds giving a red coloration when heated with phosphoric acid. Crude *O*-methylporphyroxine was separated from this mixture by preparative thin-layer chromatography (0·75 mm silica gel G /0·01N NaOH layers; solvent system, benzene : methanol 8 : 2), a zone of Rf 0·6–0·8 being eluted. Further purification was effected by extraction of an ether solution with aqueous 10% KOH to remove phenolics, crystallisation from methanol to remove the bulk of the narcotine, and countercurrent distribution (20 tubes) between chloroform and 2% aqueous tartaric acid. The material remaining after these treatments, some of which were repeated several times, was freed from residual traces of narcotine by repeated preparative thin-layer chromatography (0·25 mm silica gel G /0·01N NaOH layers; solvent system, benzene : ethyl acetate 75 : 25). A zone between Rf 0·1 and 0·3 was eluted, evaporation affording 2 mg of a light brown gum which could not be crystallised. It was shown to be homogeneous by thin-layer chromatography in six solvent systems (see Table 1), and to be identical with *O*-methylporphyroxine by thin-layer chromatography, colour reaction, infrared and ultraviolet spectra.

Authentic *O*-methylporphyroxine was prepared by methylation of porphyroxine (230 mg) with excess diazomethane in ether : ethanol at 0° for 6 hr. The

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF *O*-METHYLPORPHYROXINE ON SILICA GEL G : 0·01 N NaOH LAYERS

| System | Rf | $R_{porphyroxine}$ | $R_{narcotine}$ |
|---|---|---|---|
| Benzene : methanol 8 : 2 | 0·70 | 1·3 | 1·0 |
| Benzene : diethylamine 95 : 5 | 0·68 | 2·1 | 1·0 |
| Benzene : ethyl acetate 75 : 25 | 0·23 | 1·3 | 0·72 |
| Benzene : ethyl acetate : diethylamine 90 : 5 : 5 | 0·59 | 2·4 | 1·0 |
| Cyclohexane : chloroform : diethylamine 5 : 4 : 1 | 0·57 | 2·5 | 0·98 |
| Cyclohexane : diethylamine 8 : 2 | 0·24 | 1·8 | 0·92 |

Spray reagents: porphyroxine and *O*-methylporphyroxine, 12% aqueous phosphoric acid; narcotine aqueous potassium iodoplatinate (Genest & Farmilo, 1963).