The effect of the $\beta$-adrenoceptor blocking agent propranolol (0·1 $\mu$g/ml) was determined by administering it to the bath 20 min before terbutaline or orciprenaline. The amounts of drugs are expressed as the bases.

Twelve preparations from nine rats were used for the calculation of relaxing potency (ED50) of terbutaline and orciprenaline [terbutaline = 1·9 ($\pm$0·1 s.d.) $\times$ orciprenaline]. Slightly more than half the total number of preparations were discarded because of variation in the response to carbachol, or too much spontaneous activity, or because less than three dose-levels of the $\beta$-adrenoceptor stimulating compounds were evaluated.

Propranolol completely prevented the effect of both drugs given in doses that otherwise produced (60–80%) inhibition of the carbachol-induced contractions. A typical dose-response curve of the effects on the same preparation is shown in Fig. 1.

Terbutaline is seen to be about twice as potent as orciprenaline in relaxing the rat uterus. This ratio is similar to that found in the lung for the two compounds by Persson & Olsson (1970) who also reported orciprenaline to be more active than terbutaline on the heart (inotropic and chronotropic activity). The finding that terbutaline is more potent than orciprenaline on uterus is not surprising since Lands, Ludena & Buzzo (1967) have characterized the $\beta$-adrenoceptors in uterus to be of the same type ($\beta_2$-receptors) as in the lung and differing from the $\beta$-adrenoceptors in the heart ($\beta_1$-receptors).

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## α-Methyltryptophan increases 5-hydroxytryptamine-like material in rat brain

Previous reports from this laboratory (Sourkes, Missala & Papeschi, 1969; Sourkes, Missala & Oravec, 1970) indicated that α-methyltryptophan (AMTP) induced a decrease of tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the brain of rats. Because AMTP had been found to increase tryptophan pyrrolase activity in the liver (Sourkes & Townsend, 1955), the hypothesis was put forward that the effect on brain 5-HT was mediated by an increased flow of tryptophan in the kynurenine pathway with consequent decreased availability of substrate for 5-HT synthesis. However, the decrease of the material estimated as 5-HT after administration of AMTP was not dose-dependent; AMTP itself may also be converted to α-methyl-5-HT (AM-5-HT) in the brain, as has been reported for α-methyl-5-hydroxytryptophan (Lahti & Platz, 1969). For these reasons we re-investigated the problem by using a different method to estimate 5-HT and 5-HIAA.

Previously 5-HT was estimated according to Snyder, Axelrod & Zweig (1965) (butanol extraction-ninhydrin condensation) and 5-HIAA by the method of Giacalone & Valzelli (1966) (butyl acetate extraction-3N HCl fluorescence). I have now compared the results obtained with these methods with results obtained by the method of Ahtee, Sharman & Vogt (1970), which is based on the sequential separation of 5-HT and 5-HIAA from the same sample by column chromatography on Amberlite CG-50, type I, and Sephadex G-10. The fluorescence in 3N HCl of both compounds was read in an Aminco-Bowman spectrophotofluorometer (excitation: 295 nm;

emission: 540 nm). Male, albino, Sprague-Dawley rats, of 150–200 g, were injected intraperitoneally with 50 mg/kg of DL-AMTP and killed 16 h later. The combined butanol–butyl acetate procedure showed a slight and not significant decrease of brain 5-HT concentration, with a marked and highly significant decrease of 5-HIAA. The latter finding was confirmed by the Amberlite-Sephadex method; in contrast, the material estimated as 5-HT in this method was significantly increased by over 50% (Table 1). A dose curve was then made by measuring 5-HT and 5-HIAA in the brain of rats 6 h after the injection of AMTP, by the Amberlite-Sephadex method. Whereas 5-HIAA showed a dose-dependent decrease, the "apparent" 5-HT was slightly decreased with the lowest, but increased with the highest AMTP doses (Table 2).

Table 1. *The effect of* DL-*AMTP on the concentration of 5-HT and 5-HIAA in the brain of the rat by different methods.*

| Method | Treatment | No | Time (h) | 5-HT* (ng/g) | P** | 5-HIAA* (ng/g) | P** |
|---|---|---|---|---|---|---|---|
| (1) Butanol–butyl acetate extraction | Saline | 4 | 16 | 584 ± 41 | — | 521 ± 31 | — |
| | AMTP, 50 mg/kg | 5 | 16 | 542 ± 8 | NS | 133 ± 6 | <0·001 |
| (2) Amberlite–Sephadex chromatography | Saline | 12 | 16 | 431 ± 11 | — | 350 ± 49 | — |
| | AMTP, 50 mg/kg | 12 | 16 | 675 ± 41 | <0·001 | 123 ± 18 | <0·001 |

* = Mean ± s.e.
** = *t*-test, difference from controls.

Table 2. *Dose curve of the effect of AMTP on brain 5-HT and 5-HIAA, 6 h after the injection, by the Amberlite-Sephadex method.*

| Treatment | No | 5-HT* (ng/g) | P** | 5-HIAA* (ng/g) | P** |
|---|---|---|---|---|---|
| Saline | 4 | 423 ± 28 | — | 432 ± 33 | — |
| AMTP, 12·5 mg/kg | 4 | 370 ± 10 | NS | 239 ± 7 | <0·005 |
| AMTP, 25 mg/kg | 4 | 380 ± 10 | NS | 273 ± 38 | <0·02 |
| AMTP, 50 mg/kg | 4 | 571 ± 20 | <0·01 | 196 ± 12 | <0·001 |
| AMTP, 100 mg/kg | 4 | 650 ± 74 | <0·05 | 177 ± 32 | <0·005 |

* = Mean ± s.e.
** = *t*-test, difference from controls.

Table 3. *The effect of AMTP on 5-HT and 5-HIAA concentrations in the intestine of the rat and its interaction with yohimbine, by the Amberlite-Sephadex method.*

| Treatment | No | Time (h) | 5-HT* (μg/g) | P** | 5-HIAA* (ng/g) | P** |
|---|---|---|---|---|---|---|
| Saline | 4 | — | 5·314 ± 0·107 | — | 352 ± 26 | — |
| AMTP, 50 mg/kg | 4 | 16 | 4·413 ± 0·161 | <0·01 | 302 ± 30 | NS |
| Yohimbine, 5 mg/kg | 3 | 2 | 6·475 ± 0·235 | <0·01 | 317 ± 13 | NS |
| Yohimbine 5 mg/kg + AMTP, 50 mg/kg | 4 | 2<br>16 | 4·932 ± 0·194 | NS | 267 ± 18 | <0·05 |

* = Mean ± s.e.
** = *t*-test, difference from controls.

The increase of 5-hydroxyindole material, estimated as 5-HT in the second method, cannot be accounted for by interference of AMTP itself; indeed, in our experimental conditions, pure solutions of AMTP yielded a fluorescence intensity that was 1/5000 that yielded by authentic 5-HT. Even supposing that AMTP is distributed evenly in the body and that the ratio of AMTP: 5-HT in the brain is in the order of 100 after injection of 50 mg/kg of AMTP, the interference of AMTP in the fluorescence readings could not have exceeded 2%. Thus, it appears that a compound is formed in the brain after injections of AMTP that is retained on the Amberlite columns and estimated as 5-HT, but that does not (or only slightly) interfere with the butanol-ninhydrin method. This compound is likely to be AM-5-HT which yields almost the same fluorescence intensity as 5-HT and has been isolated on paper chromatography from the brain of rats treated with AMTP (Roberge, Sourkes & Missala, in the press). On the other hand, the consistent decrease of brain 5-HIAA may stem from two possible effects of AMTP: (1) inhibition of monoamine oxidase, as the AM-5-HT formed would not be a substrate for this enzyme. (2) A decreased turnover of brain 5-HT due to the activation of liver pyrrolase; although the material estimated as 5-HT by the Amberlite-Sephadex method is increased, the "true" 5-HT may very well be decreased after AMTP.

The increase of 5-HT-like material occurs in the brain only; in fact AMTP (50 mg/kg, i.p.) decreased significantly 5-HT in the small intestine of the rat 16 h after the injection, and antagonized the increase of 5-HT induced by yohimbine (Papeschi, Sourkes & Youdim, 1971), as measured by the Amberlite-Sephadex method (Table 3).

Because of the possibility that AM-5-HT formed from AMTP acts as a false neurotransmitter, and because the synthesis of "true" 5-HT may be decreased after AMTP, I studied the effects of repeated injections of AMTP on the behaviour of the rat. Four animals were injected (100 mg/kg, i.p.) every 3 h (total: 400 mg/kg) on the first day; at 24, 27 and 30 h they received 100, 200 and 500 mg/kg of the drug, respectively. The behaviour was observed every hour for 5–10 min on both experimental days, up to 36 h after the first injection. Motor behaviour was also tested with the "vertical wire" and "four-corks" tests; rectal temperature was recorded with an electronic thermometer. No significant alteration of social or motor behaviour was observed at any time after AMTP, despite the marked changes in 5-HT metabolism that have been reported, and certainly no sedation was apparent. A slight but persistent hypothermia was instead observed (average 1° below the controls).

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