J. Pharm. Pharmac., 1971, **23**, 606–611 *Received January* 5, 1971

Effect of amphetamine on the uptake, release and effectiveness of xylocholine in the guinea-pig vas deferens

H. G. DEAN AND I. E. HUGHES*

Department of Pharmacology, The Medical School, Thoresby Place, Leeds LS2 9NL, Yorkshire, U.K.

Amphetamine sulphate will both reverse and prevent the adrenergic neuron blocking action of xylocholine (TM10 bromide) on the response of the guinea-pig isolated vas deferens to transmural electrical stimulation. A concentration of amphetamine sulphate capable of reversing the effect of xylocholine does not produce a significant reduction in the tissue concentration of ^{14}C-TM10 iodide in the vas deferens. Although amphetamine reduces the rate of uptake of xylocholine, it does not prevent uptake. Comparisons of tissue concentrations with the degree of blockade produced in the normal and the amphetamine-treated vas deferens suggest that if the actions of amphetamine are to be accounted for entirely by displacement of xylocholine or by changes in uptake of xylocholine, only a very small percentage of the total tissue content of xylocholine can be involved in the production of its effects.

It is well established that the adrenergic neuron blocking action of a variety of compounds can be prevented by the prior administration of amphetamine and that this agent will also reverse an established adrenergic neuron blockade (Wilson & Long, 1960; Day, 1962; Follenfant & Robson, 1970). Tissue concentrations of *NNN*-trimethyl-2-(2,6-xylyloxy)ethylammonium iodide (TM10 iodide) achieved during the establishment of adrenergic neuron blockade in a tissue receiving mainly adrenergic innervation have now been measured to see if these tissue concentrations were modified by the administration of amphetamine in doses known to be effective in reversing or preventing adrenergic neuron blockade.

METHODS

NN-*dimethyl*-N-*methyl*-[^{14}*C*]-2-(2,6-*xylyloxy*)*ethylammonium iodide* (^{14}*C-TM*10 *iodide*). Methyl[^{14}C]iodide (0·5 mCi, 0·009 mmol) was condensed in a vacuum onto *NN*-dimethyl-2-(2,6-xylyloxy)ethylamine (0·42 mmol) in dry acetone (5 ml) and the mixture was allowed to stand at room temperature for 24 h. Methyl iodide (0·8 mmol) was then condensed onto the reaction mixture which was allowed to stand at room temperature (20°) for a further 24 h. The acetone and excess methyl iodide were removed in a vacuum and a white crystalline deposit of ^{14}C-TM10 iodide remained (0·42 mmol: m.p. 226–228°: specific activity, 1·31 mCi/mmol). [A sample of this material prepared under the same conditions but omitting the addition of methyl [^{14}C] iodide had a m.p. of 229–230°. Found: C, 46·35; H, 6·4; N, 4·4%. $C_{13}H_{22}INO$ requires C, 46·6; H, 6·61; N, 4·17%.]

* To whom requests for reprints should be sent.

Thin-layer chromatography of the ^{14}C-TM10 iodide followed by scanning of the plates in a Tracerlab Scanner and by autoradiography showed a single spot in the t.l.c. systems in Table 1.

Preparation of tissues. Vasa deferentia were removed from freshly-killed guinea-pigs (weight, 300–600 g) and transferred to Krebs solution (NaCl, 6·9; KCl, 0·35; $CaCl_2.6H_2O$, 0·65; $MgSO_4.7H_2O$, 0·28; K_2HPO_4, 0·16; $NaHCO_3$, 2·1; glucose, 2·0 g/litre: gassed with 5% carbon dioxide in oxygen) at room temperature.

Transmural stimulation. Vasa deferentia were suspended in Krebs solution at 37° and were allowed to equilibrate for 0·25 h. Electrical stimulation (rectangular wave pulses, 50/s, 0·5 ms duration, supramaximal voltage) was applied for 7 s every 0·25 h (or as required) through transmural electrodes (Birmingham & Wilson, 1963) and contractions were recorded on a Heathkit chart recorder via an isotonic transducer. Two or three control responses were elicited from the tissue before any drugs were added to the bath and all responses were expressed as a percentage of these control responses. Multiple determinations of the effect of the several treatments employed were made on different vasa deferentia and means and standard errors of the size of the response to transmural stimulation at each application were calculated.

Table 1. *Thin-layer chromatography systems used and the approximate R_F values of the single spots obtained on chromatography of ^{14}C-TM10 iodide.*

Plate	Solvent	R_F
0·25 mm silica gel G (Stahl)	Dry acetone–water (20:30) containing 1% w/total volume NaBr	0·55
”	Ethanol–water (50:2) containing 1% w/total volume NaBr	0·3
0·25 alumina for t.l.c. (Hopkin & Williams)	Cyclohexane–chloroform–glacial acetic acid–isopropanol (32:32:4:6)	0·3
”	Cyclohexane–chloroform–glacial acetic acid–ethanol (32:32:8:8)	0·55
”	Cyclohexane–chloroform–glacial acetic acid (45:45:11)	0·45

Uptake of TM10 iodide. Vasa deferentia were incubated at 37° in Krebs solution gassed with water-saturated 5% carbon dioxide in oxygen and containing 1·91 μg/ml ^{14}C-TM10 iodide with or without the presence of 10 μg/ml amphetamine sulphate. On completion of the incubation (which was for up to 1·5 h) the tissues were removed from the solution, blotted dry on filter paper, weighed on a torsion balance, placed on small ($\approx$150 mg) squares of filter paper and allowed to air-dry for 1–3 h. The tissue [^{14}C] content was determined by burning the tissue and filter paper in a Packard Sample Oxidizer (model 305) and counting the radioactivity of the resulting solutions in a Packard Liquid Scintillation Spectrometer (model 3320). Results, corrected for counting efficiency (using external standardization), are expressed as ng ^{14}C-TM10 iodide per g wet weight tissue (mean and standard error). Results have not been corrected for recovery of ^{14}C from the combustion process which was 84·9 $\pm$ 0·5% as determined by combustion of known amounts of ^{14}C-TM10 iodide spotted on to Kleenex tissue.

Release of ^{14}C-TM10 iodide. Tissues were incubated with 1·91 μg/ml ^{14}C-TM10 iodide for 1·5 h as described above and were then removed from the radioactive

solution, washed for 3 s in Krebs solution at 37° and placed in tissue baths at 37° containing either drug-free Krebs solution or Krebs solution plus (±)-amphetamine sulphate (10 μg/ml). The tissue baths were drained and refilled with fresh solutions every 0·25 h and tissue [^{14}C] content was determined at suitable intervals as described above.

The following drugs were used: (±)-amphetamine sulphate, cinchocaine hydrochloride, *NN*-dimethy-*N*-methyl-[^{14}C]-2-(2,6-xylyloxy)ethylammonium iodide ^{14}C-TM10 iodide and *NNN*-trimethyl-2-(2,6-xylyloxy)ethylammonium bromide (TM10 bromide: xylocholine) and all concentrations are expressed in terms of these salts.

Tests for significance of difference were performed using Students *t*-test.

RESULTS

Transmural stimulation. Transmural stimulation of vasa deferentia as described and in the absence of any drugs, elicited reproducible responses from the tissue for at least 6 h (5 exp). In the presence of 1·64 μg/ml of TM10 bromide (xylocholine) (equivalent to 1·91 μg/ml ^{14}C-TM10 iodide) a blockade of the effects of transmural stimulation was produced which became progressively greater over the 1·5 h during which the drug was allowed to remain in contact with the tissue (Fig. 1). The response elicited by transmural stimulation 1·5 h after the addition of the drug to the tissue bath was 28·2 ± 6·6% of the control response. Immediately after this response had been obtained, the bath was drained and refilled with fresh drug-free Krebs solution and this was repeated every 0·25 h for 1 h during which time the

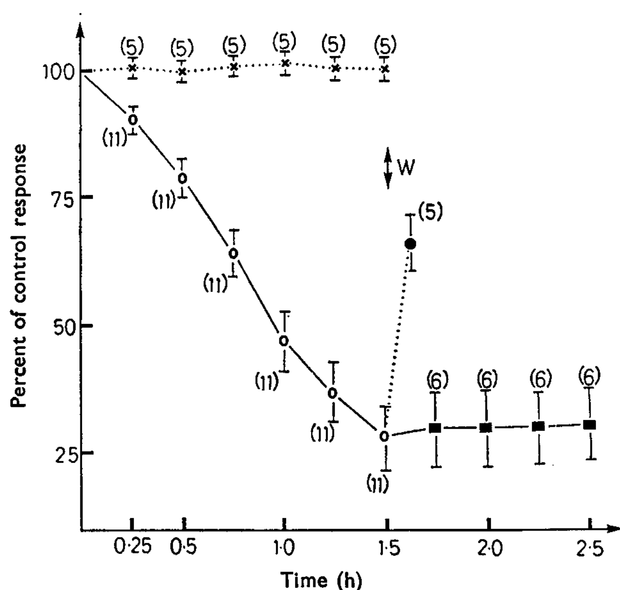

FIG. 1. Showing the means and standard errors of the responses (percentage of control response) of several (figures in parentheses) guinea-pig vasa deferentia to transmural stimulation (rectangular wave pulses, 50/s, 0·5 ms duration, supramaximal voltage: 7 s every 0·25 h) plotted against the time (h) the tissue was exposed to the following treatments: × . . . ×, TM10 bromide (1·64 μg/ml) and amphetamine sulphate (10 μg/ml) administered simultaneously. ○——○ TM10 bromide (1·64 μg/ml) alone. At W, the tissue bath was drained and refilled with fresh Krebs solution (■——■) or with Krebs solution plus 10 μg/ml amphetamine sulphate (○ . . . ●) and this process was then repeated every 0·25 h. In the latter case transmural stimulation was applied 7 min after addition of the amphetamine sulphate.

tissue was stimulated transmurally every 0·25 h as usual. Little reversal of the blockade was produced in this 1 h period; the response was not significantly different ($P > 0{\cdot}9$) from that obtained before the washing process started (Fig. 1).

The addition of amphetamine sulphate to the tissue bath immediately after the first wash did produce a significant reversal of the blockade ($P < 0{\cdot}01$). The response to transmural stimulation 7 min after the addition of amphetamine being 65·9 ± 5·0% of the control response. This reversal of the blocking action of TM10 bromide by amphetamine was fast in onset and in each of 5 additional experiments a significant reversal of the blockade was seen on application of transmural stimulation 30 s after the addition of amphetamine.

When 1·64 μg/ml TM10 bromide and 10 μg/ml amphetamine sulphate were administered to the tissue simultaneously at the start of the experiment, no blockade of the response to transmural stimulation developed within 1·5 h, after which time the experiment was terminated (Fig. 1). At this time the response to transmural stimulation was 100·8 ± 0·6% of the control response and was not significantly different ($P > 0{\cdot}3$) from the response of the tissue at this time in the absence of all drugs.

The blockade of the response to transmural stimulation produced by 1·5 μg/ml cinchocaine hydrochloride was not prevented (2 exps) or reversed (3 exps) by the administration of 10 μg/ml of amphetamine sulphate.

Tissue concentrations of ^{14}C-TM10 iodide. On incubation of vasa deferentia with 1·91 μg/ml ^{14}C-TM10 iodide (equivalent to 1·64 μg/ml TM10 bromide) there was a fast initial uptake of ^{14}C-TM10 iodide during the first 0·25 h of the incubation and thereafter tissue concentrations continued to rise for at least 1·5 h (Table 2 and Fig. 2). Immediately after this time, tissues were removed from the radioactive solution and transferred to tissue baths containing drug-free Krebs solution or Krebs solution containing 10 μg/ml of amphetamine sulphate. After 7 min further incubation in these solutions, the tissue content of the two groups of vas deferens were 8104 ± 594 and 7963 ± 369 ng ^{14}C-TM10 iodide/g tissue respectively, and were not significantly

Table 2. *Tissue concentrations of ^{14}C-TM10 iodide* (ng/g) *in guinea-pig vasa deferentia (means ± standard errors) during incubation in Krebs solution at* 37° *containing* 1·91 μg/ml *^{14}C-TM10 iodide with or without* 10 μg/ml *amphetamine sulphate.* The figures in parentheses indicate the number of vasa deferentia used for each determination. Levels of significance were calculated using Students *t*-test.

Duration of incubation (h)	^{14}C-TM10 iodide ng/g tissue	
	Without amphetamine	With amphetamine
0·25	3033·3 ± 402·9 (6)	1338·3 ± 60·0** (6)
0·5	4570·8 ± 433·7 (8)	1967·3 ± 121·3*** (6)
1·0	5931·2 ± 513·6 (8)	3044·5 ± 344·4** (6)
1·5	8870·8 ± 582·5 (10)	3941·2 487·9*** (6)

** = $P \leqslant 0{\cdot}01$.
*** = $P \leqslant 0{\cdot}001$.

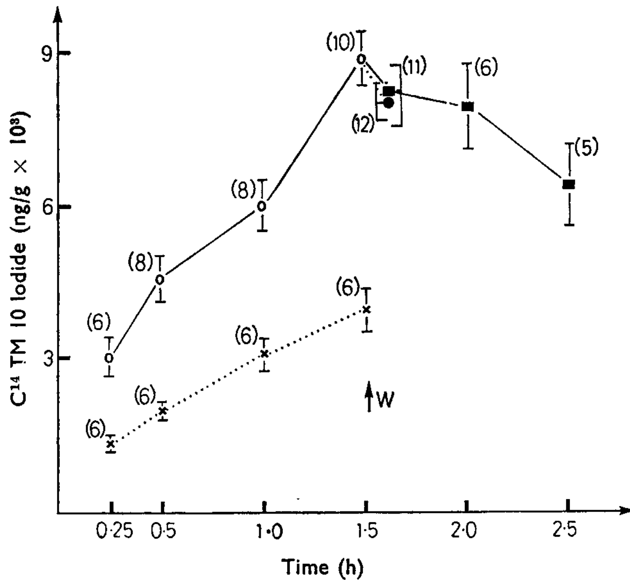

FIG. 2. Means and standard errors of the tissue concentrations (ng/g) of ^{14}C-TM10 iodide attained in guinea-pig vas deferens incubated at 37° in Krebs solution containing 1·91 μg/ml ^{14}C-TM10 iodide alone (○——○) or plus 10 μg/ml amphetamine sulphate (× . . . ×). At W, the tissues incubated in the absence of amphetamine were transferred to a tissue bath containing fresh Krebs solution (■——■) or Krebs solution containing 10 μg/ml amphetamine sulphate (. . . ●). The figures in parentheses indicate the number of tissue estimations contributing to each point.

different ($P > 0·3$). Some vasa deferentia were allowed to remain in drug-free Krebs solution for up to 1 h during which time no marked fall in the tissue content of ^{14}C-TM10 iodide was observed.

Incubation of vasa deferentia with 1·19 μg/ml ^{14}C-TM10 iodide in the presence of 10 μg/ml of amphetamine sulphate also produced a steady increase in the tissue content of ^{14}C-TM10 iodide over 1·5 h (Table 2). The tissue concentrations attained however were only $\approx$30–50% of those obtained during incubation with ^{14}C-TM10 iodide alone at each incubation time investigated (Table 2).

DISCUSSION

The results show that the slowly-developing blockade produced by TM10 bromide (xylocholine) of the response of the guinea-pig vas deferens to transmural stimulation can be completely prevented by the simultaneous administration of amphetamine sulphate (Fig. 1).* Determinations of the tissue content of vasa deferentia incubated with an equivalent concentration of ^{14}C-TM10 iodide show that there is a slow accumulation of ^{14}C-TM10 iodide in the tissue and that this is not prevented by amphetamine sulphate. Amphetamine does however reduce the rate of accumulation and the magnitude of the tissue concentrations attained in a given time (Fig. 2). After 1·5 h incubation in its presence tissue concentrations were approximately equal to those attained after 22 min incubation with ^{14}C-TM10 iodide alone. At

* This is unlikely to be due to any "direct" action of amphetamine on the tissue since the actions of cinchocaine hydrochloride were not modified by amphetamine.

this time, in the absence of amphetamine, the response of the tissue to transmural stimulation was reduced to about 85% of the control response but in its presence no corresponding blockade was seen. These results suggest that though the reduction in uptake of ^{14}C-TM10 iodide produced by amphetamine may contribute to a reduction in the effects of TM10 bromide, this effect of amphetamine is insufficient in itself to account completely for the reduction in TM10 bromide effects.

When vasa deferentia that had been treated with TM10 bromide for 1·5 h were then treated with amphetamine, a marked reversal of the blockade of the response to transmural stimulation was produced. This treatment did not however produce any significant change in the total tissue content of ^{14}C-TM10 iodide. Amphetamine cannot therefore displace from the tissue much of the total tissue content of ^{14}C-TM10 iodide. Since the action of amphetamine under these conditions can be demonstrated as soon as 30 s after administration, it seems unlikely that a relocation of any major portion of the ^{14}C-TM10 within the tissue could take place within the short time period.

It seems likely therefore that neither prevention of uptake nor displacement of TM10 can entirely account for the ability of amphetamine to prevent or to reverse the blocking action of TM10 in the guinea-pig vas deferens unless only a very small proportion of the total tissue content of xylocholine is involved in the production of its actions.

REFERENCES

BIRMINGHAM, A. T. & WILSON, A. B. (1963). *Br. J. Pharmac. Chemother.*, **21**, 569–580.
DAY, M. D. (1962). *Ibid.*, **18**, 421–439.
FOLLENFANT, M. J. & ROBSON, R. D. (1970). *Br. J. Pharmac.*, **38**, 792–810.
WILSON, R. & LONG, C. (1960). *Lancet*, **2**, 262.