# Methadone *N*-oxide in the urine of methadone maintenance subjects—an artifact?

Our laboratories have been active in the investigation of the disposition and metabolism of methadone in man for a number of years. During the course of these studies a relatively large number of metabolites of methadone in man has been identified (Pohland, Boaz & Sullivan, 1971; Sullivan & Blake, 1972; Sullivan, Due & McMahon 1972; Sullivan, Smits & others, 1972). However, we have not to date identified methadone *N*-oxide as a metabolite of methadone in man. Because of the recent report of Beckett, Vaughan & Essien (1972), that methadone-*N*-oxide is an important metabolite of methadone in man, we wish to record our experience in this matter.

Authentic methadone *N*-oxide was prepared and its properties investigated. We found, as did Beckett, Mitchard & Shihab (1971), that methadone-*N*-oxide is readily extractable from urine with chloroform or mixtures of benzene and chloroform and that it can be identified and quantitated by comparison t.l.c. or g.l.c. But the g.l.c. peak seen is not methadone-*N*-oxide, rather it is its thermal elimination product, 4,4-diphenyl-5-ketoheptene-2. Similarly the mass fragmentation product seen by combined gas chromatography-mass spectrometry (g.c.-m.s.) is that of the elimination product. The electron impact (70 eV) mass spectrum of 4,4-diphenyl-5-ketoheptene-2 does not contain a mass ion, but the two peaks of major intensity at $m/e$ 129 and $m/e$ 207 are characteristic and serve to identify the compound.

Urine collections were made over 24 h from six men who were receiving daily maintenance doses of $\pm$ methadone HCl of 80–100 mg. Urines were immediately frozen and stored at $-10°$ in closed containers. When these samples were thawed and immediately analysed by the above procedures no methadone-*N*-oxide was detectable. For both the g.c. and the g.c.-m.s. studies, 4 ft siliconized glass columns packed with 1% W-98 (methylvinyl silicon gum rubber) on 80–100 mesh Gas-Chrom Q were used. Good separations were obtained at 165° with helium as a carrier gas.

A sample of freeze dried urine was also obtained from a subject receiving [$^{14}$C]-methadone (this urine was kindly supplied to us by Dr. E. Änggård and Dr. L. Gunne, Uppsala). Authentic *N*-oxide was added to this urine and then recovered by extraction into chloroform. Thin-layer chromatography (Beckett & others, 1971) of this extract yielded an *N*-oxide spot containing no label thus indicating the absence of methadone-*N*-oxide in the sample.

Since it has been our experience that methadone and its metabolites are chemically unstable in solution, the following experiment was performed. Samples of experimental urine, control urine to which methadone had been added and solutions of methadone in organic solvents were allowed to stand at 30° for 5 days. In all cases these samples were now found to contain substantial amounts of a material which could be identified as methadone-*N*-oxide by the criteria described above. Extracts of fresh urine from maintenance subjects were also found to develop the *N*-oxide peak upon storage at 30°.

We have concluded that methadone-*N*-oxide is probably not an important metabolite of methadone in man and that its apparent presence in urine is an artifact. This work will be described in detail shortly (Sullivan & Due, 1973).

*The Lilly Research Laboratories,*
*Indianapolis, Indiana 46206, U.S.A.*

HUGH R. SULLIVAN
SUSAN L. DUE
ROBERT E. MCMAHON

July 23, 1973

REFERENCES

BECKETT, A. H., VAUGHAN, D. P. & ESSIEN, E. E. (1972). *J. Pharm. Pharmac.*, **24**, 244.
BECKETT, A. H., MITCHARD, M. & SHIHAB, A. A. (1971). *Ibid.*, **23**, 347–352.
POHLAND, A., BOAZ, H. E. & SULLIVAN, H. R. (1971). *J. medl Chem.*, **14**, 194–197.
SULLIVAN, H. R. & BLAKE D. A. (1972). *Res. Comm. Chem. Path. Pharmac.* **3**, 467–478.
SULLIVAN, H. R. & DUE, S. L. (1973). *J. medl Chem.*, in the press.
SULLIVAN, H. R., DUE, S. L. & MCMAHON, R. E. (1972). *J. Am. chem. Soc.*, **94**, 4050–4051.
SULLIVAN, H. R., SMITS, S. E., DUE, S. L., BOOHER, R. E. & MCMAHON, R. E. (1972). *Life Sci.*, Part I, **11**, 1093–1104.

# Post-synaptic blockade of dopaminergic transmission by 6-hydroxydopamine

6-Hydroxydopamine selectively destroys adrenergic nerve terminals and may also block peripheral α-adrenoceptors (e.g. Furness, 1971). Any post-synaptic effects of 6-hydroxydopamine on dopaminergic transmission are likely to be difficult to study because of the mainly central localization of dopamine in vertebrates. The effects of 6-hydroxydopamine reported here were studied on synaptic transmission from a giant dopamine-containing cell in the left pedal ganglion of the water snail *Planorbis corneus*. This cell, discovered in freeze-dried preparations (Marsden & Kerkut, 1970) and shown to contain dopamine by biochemical methods (Berry, Cottrell, Pentreath & Powell, unpublished observations), was identified in living ganglia (Berry & Cottrell, 1973), and offers a unique opportunity to study the transmitter function of dopamine. Post-synaptic responses to stimulation of the giant cell occur in at least 20 neurons in the visceral and left parietal ganglia. Responses are excitatory, inhibitory or biphasic, and appear to result from release of dopamine (Berry & Cottrell, 1973).

When 6-hydroxydopamine ($2{\cdot}5 \times 10^{-4}$ M) was tested on inhibitory post-synaptic potentials (IPSPs) produced in post-synaptic cells by stimulation of the cell, there was a total abolition of response. The effect, which was reversible, was found to be due to a blockade of dopamine receptors; the hyperpolarizing response to applied dopamine was abolished. After the preparation had been washed for 10–60 min, there was a gradual recovery of IPSPs and concomitant recovery of the response to dopamine. There was no effect of 6-hydroxydopamine on the hyperpolarizing response to glutamate shown by the post-synaptic cells, or on depolarizing responses to 5-HT and acetylcholine. A check on the specificity of the responses was made by observing the action on spontaneous inhibitory input and on IPSPs produced by stimulating a nerve trunk. There is evidence that these IPSPs are not produced by dopamine but possibly by glutamate. They were only slightly reduced by 6-hydroxydopamine. Excitatory responses of cells in the parietal ganglion to stimulation of the giant cell were abolished by 6-hydroxydopamine together with the depolarizing response to applied dopamine. The results indicate that 6-hydroxydopamine produces specific abolition of dopaminergic transmission by blocking dopamine receptors in this snail.

*Wellcome Laboratories of Pharmacology,*
*University of St. Andrews,*
*St. Andrews, U.K.*

M. S. BERRY
G. A. COTTRELL

October 2, 1973

REFERENCES

BERRY, M. S. & COTTRELL, G. A. (1973). *Nature, New Biology*, **242**, 250–253.
FURNESS, J. B. (1971). In: *6-Hydroxydopamine and Catecholamine Neurons*. Editors: Malmfors, T. & Thoenen, H. pp. 205–214. Amsterdam & London: North-Holland Publishing Company.
MARSDEN, C. & KERKUT, G. A. (1970). *Comp. Gen. Pharmac.*, **1**, 101–116.