about 0·7 cm^2/mg wet weight, so that the TTX site density would be 33 sites/μm^2 (cf. 27 sites/μm^2 in rabbit vagus C fibres (Colquhoun *et al.*, 1972)).

Cobra toxin binding capacity was measured by saturating muscles with ^{3}H-Naja nigricollis toxin (100 nM for 7 h, followed by 3 h washing in toxin-free solution). In normal muscle the uptake of cobra toxin was $8·7 \pm 1·4$ (S.E. of mean, $n=6$) fmole/mg wet: 7–14 days after denervation it increased to $50·9 \pm 2·8$ ($n=6$) fmole/mg wet. The increase after denervation, 42 fmole/mg wet, is thus about 10 times greater than the TTX binding capacity of the normal muscle. It is thus unlikely that denervation changes can be explained simply by interconversion of channels from one type to another.

This work was supported in part by a grant NS08304 from the U.S.P.H.S. We are grateful to Dr. J.-P. Changeux for supplying the purified α-toxin.

REFERENCES

COLQUHOUN, D., HENDERSON, R. & RITCHIE, J. M. (1972). The binding of labelled tetrodotoxin to non-myelinated nerve fibres. *J. Physiol., Lond.* (in press).

LEE, C. Y., TSENG, L. F. & CHIU, T. H. (1967). Influences of denervation on localization of neurotoxin from elapid venoms in rat diaphragm. *Nature*, **215**, 1177–1178.

LESTER, H. A. (1970). Postsynaptic action of cobra toxin at the myoneural junction. *Nature*, **227**, 727–728.

MILEDI, R. (1960). The acetylcholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol., Lond.*, **151**, 1–23.

REDFERN, P. & THESLEFF, S. (1971). Action potential generation in denervated rat skeletal muscle. *Acta physiol. Scand.*, **82**, 70–78.

Studies on 5-hydroxytryptamine receptors of neurones from *Hirudo medicinalis*

P. A. SMITH and R. J. WALKER*

Department of Physiology and Biochemistry, Southampton University, Southampton SO9 5NH

5-Hydroxytryptamine (5-HT) has been shown to be present in leech Retzius cells (Kerkut, Seddon & Walker, 1967) and to have an inhibitory action on these cells (Kerkut & Walker, 1967) but the ionic mechanism for this inhibition has not been determined. Intracellular recordings were made from Retzius cells in isolated ganglion chain preparations of the leech *Hirudo medicinalis* using glass microelectrodes filled with molar potassium acetate. The potentials were amplified and displayed on a Tektronix 502A oscilloscope and permanent traces obtained using an A.E.I. pen oscillograph. The Ringer used had the following composition: NaCl 115 mM; KCl 4 mM; $CaCl_2$ 2mM; Tris-chloride buffer 10 mM; glucose 10 mM; pH 7·4.

Results from changing either external or internal chloride levels suggested that the major ion involved in 5-HT inhibition was chloride. The 5-HT response was measured in normal and in 50 mM chloride Ringer, chloride being replaced by acetate. From the Nernst equation the internal chloride concentration was calculated to be 9 mM (mean of five experiments), giving a chloride equilibrium potential of about 65 mV. Changing either external sodium or potassium levels or using a Ringer containing 20 mM $MgCl_2$ had no effect on the 5-HT response. Strychnine also had an inhibitory effect on Retzius cells and this inhibition was also mediated through a change in chloride permeability (Prichard, 1971). However, desensitization experiments and the use of a specific 5-HT antagonist, mianserin, suggested that 5-HT and strychnine acted on different receptors.

Equipotent molar ratios were determined for analogues of 5-HT, the results being a mean of five experiments. These studies indicated the importance of the hydroxyl group of 5-HT for maximum 5-HT like activity. Tryptamine was 120 (range 38–200) times less active than 5-HT. Substitution of the hydroxylgroup for either chloro or methoxy reduced the potency 55 (range 9–140) and 77 (range 21–285) times respectively. The addition of a methyl group on the alpha carbon of the side chain of 5-HT or the

addition of either one or two methyl groups on the terminal nitrogen of the side chain slightly reduced the potency, the potency ratio for alpha-methyl-5-HT, N-methyl-5-HT and bufotenine being 3 (range 2–5), 6·5 (range 4–9) and 10 (range 2–26) respectively compared with 5-HT.

It is concluded that 5-HT acts directly on a specific 5-HT receptor on the Retzius cell membrane to increase chloride conductance.

REFERENCES

KERKUT, G. A., SEDDON, C. B. & WALKER, R. J. (1967). Cellular localization of monoamines by fluorescence microscopy in *Hirudo medicinalis* and *Lumbricus terrestris*. *Comp. Biochem. Physiol.*, **21**, 687–690.

KERKUT, G. A. & WALKER, R. J. (1967). The action of acetylcholine, dopamine and 5-hydroxytryptamine on the spontaneous activity of the cells of Retzius of the leech, *Hirudo medicinalis*. *Brit. J. Pharmac.*, **30**, 644–654.

PRICHARD, J. W. (1971). The effect of strychnine on the leech Retzius cell. *Neuropharmacology*, **10**, 771–774.

Some characteristics of the uptake process in the isolated blood perfused cat spleen resistant to desmethylimipramine and 17-β-oestradiol

A. G. H. BLAKELEY, G. POWIS and R. J. SUMMERS*

Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ

It has recently been shown that a process exists in the isolated blood perfused cat spleen which, after complete inhibition of $Uptake_1$ and $Uptake_2$ by desmethylimipramine (DMI) ($3{\cdot}3\times10^{-5}$M) and 17-β-oestradiol (17βO) ($1{\cdot}8\times10^{-4}$M), takes up (−)-noradrenaline (NA) (Blakeley, Powis & Summers, 1972).

Uptake by this process from 1 μg pulses of ^{3}H-NA has been measured during perfusion of spleens with different media. Two pulses were given while the spleen was perfused with blood. The uptake inhibitors DMI and 17βO were given between the pulses. Under these conditions uptake of the second pulse was $90{\cdot}62\%\pm1{\cdot}53$ (S.E. of mean) of the first. The red cells were removed by gentle centrifugation and the spleen perfused with plasma. Uptake of the next pulse was reduced to $54{\cdot}36\pm5{\cdot}52\%$ ($n=5$, $P<0{\cdot}001$). The red cells were then resuspended in Krebs–Henseleit solution containing DMI ($3{\cdot}3\times10^{-5}$M) and 17βO ($1{\cdot}8\times10^{-5}$M—an almost saturated solution). This medium restored uptake to $86{\cdot}84\pm6{\cdot}88\%$ ($n=5$, $P>0{\cdot}2$), not significantly different from that observed during blood perfusion. Subsequent perfusion with plain Krebs–Henseleit solution was followed by a drop in uptake to $49{\cdot}29\pm6{\cdot}65\%$ ($n=5$, $P<0{\cdot}001$).

After the injection of a pulse in the presence of DMI and 17βO about 75% of the NA appeared unchanged in the venous blood during the subsequent 3 min, together with a small quantity (<1%) of normetanephrine. Smaller amounts (about 6%) of NA and normetanephrine (<0·5%) came over in the next 12 minutes. Only trace amounts (<0·1%) of MAO metabolites were found in blood. Fifteen minutes after the pulse the spleen was homogenized and examined for retained NA and metabolites. About half the pulse retained was present as unchanged NA and the rest consisted of equal parts of MAO and MAO/COMT metabolites. No normetanephrine was found in the spleen.

Unlike $Uptake_1$ (Kirpekar & Puig, 1971), uptake of pulses was inhibited by nerve stimulation. ^{3}H-NA pulses were accompanied by a volume marker, ^{14}C-polyethylene glycol. Stimulation of the splenic nerves at 3 Hz produced only small vascular and capsular responses of the spleen and produced no effect on flow judged from the pattern of overflow of the volume marker, yet uptake was reduced to $66{\cdot}46\pm1{\cdot}89\%$ of controls ($n=3$, $P<0{\cdot}05$).