Measurements of noradrenaline overflow and dopamine-β-hydroxylase activity in superfused preparations

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The nervously evoked vascular release of sympathetic neurotransmitter is accompanied by dopamine-β-hydroxylase (DBH) release into the extracellular space, (Axelrod, 1972), it has also been shown that the amount of both released neurotransmitter noradrenaline (NA) and DBH are directly proportional (Weinshilboum, Thoa, Jhonson, Kopin & Axelrod, 1971; Cubeddu, Barnes, Langer & Weiner, 1974).

Although these findings have been reported for *in vivo* preparations and *in vitro* using [^{3}H]-NA loading for estimation of NA, few results have been presented using endogenously derived NA from superfused preparations. Accordingly a method is presented in which NA concentration and DBH activity can be measured simultaneously. The combination of superfusion method and perimural stimulation is achieved by attaching the tissue on a specially constructed perspex holder; stimulation being effected by two platinum electrodes; the gap between the electrodes and the tissue being made sufficiently wide to allow the muscle to contract freely but for the superfusate to be retained by capillary action thus making it the medium for electrical stimulation. In these studies rat anococcygeus muscle and vas deferens preparations were examined.

DBH activity was measured using the method of Kato, Kuzuya & Nagatsu (1974) based on modifications described by Algate & Leach (1978). The optimal Cu^{2+} concentration for rendering the endogenous inhibitors inactive was found to be 16 μM and the rate of octopamine production from its tyramine substrate was linear up to 3 hours. Two hours incubation was used throughout the experiments. Boiled samples were used as blanks and octopamine was used as an internal standard. After octopamine oxidation to p-hydroxybenzaldhyde and extraction, the reaction product was measured by differential spectrophotometry at 333 and 360 nm.

Noradrenaline was separated using Sephadex G10 (Westerink & Korf, 1976) supported in a Pasteur pipette column; after use the column was regenerated by ammonium hydroxide 0.01 N and formic acid 0.01 N. The tissue superfusate was first adjusted to pH 2.00 with perchloric acid 5% before addition to the column. Elution was achieved by 2 ml formic acid 0.01 N and 1 ml phosphate buffer pH 8.5 0.005 M.

Spectrophotofluorometric analysis was made according to Kher, Lindguist & Carlsson (1976) with modifications, in which $K_3Fe(CN_6)$ 80 mg% was used to oxidise NA and the pH adjusted to 6.5 by phosphate buffer. The fluorescence was read at 380 nm and 500 nm. This method to be demonstrated has proved to have several advantages over the use of alumina and ion exchange. Sephadex G10 can be used for several months without detectable changes in the recovery which is higher than 85% and the blank readings are consistently small. It has also been proved that Sephadex can be used to separate noradrenaline metabolites (Westerink & Korf, 1977).

Results will be presented of the effects on the preparations of some drugs which modify sympathetic mechanisms. Phenoxybenzamine, yohimbine, phentolamine and desmethylimpramine significantly increase both NA overflow and DBH activity in the superfusate after tissue stimulation and the methods are sensitive enough to be applied to superfusate analysis, for routine work and teaching purposes.

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