*Br. J. Pharmac.* (1978), **64**, 3–5.

# ADRENAL ORIGIN OF PLASMA CATECHOLAMINES AFTER DECAPITATION: A STUDY IN NORMAL AND DIABETIC RATS

B.A. BERKOWITZ & R.J. HEAD

Roche Institute of Molecular Biology, Nutley, New Jersey 07110, U.S.A.

The concentrations of catecholamines and the activities of dopamine-β-hydroxylase were measured in blood obtained from decapitated diabetic and aged-matched control rats. The activity of dopamine-β-hydroxylase in blood from diabetic rats was much greater (5 fold) than that seen for control rats. For both diabetic and control rats, decapitation was accompanied by an increase in levels of adrenaline and noradrenaline with no change in the activity of plasma dopamine-β-hydroxylase. The results are consistent with a predominantly adrenal origin of catecholamines and extra-adrenal origin of dopamine-β-hydroxylase. The high activity of dopamine-β-hydroxylase in diabetes indicates either an increased activity of the sympathetic nervous system or changes in dopamine-β-hydroxylase turnover.

**Introduction** Biochemical approaches to estimation of the activity of the sympathetic nervous system focus on the measurement of the neurotransmitter catecholamines (CA) and the activity of the enzymes responsible for their synthesis. Analysis of circulating CAs can provide a net assessment of sympathetic nervous system activity (Cryer, 1976) and has the distinct advantage of an accessible compartment from which to sample. A second marker of sympathetic nervous system activity which has been used is the activity in serum of dopamine-β-hydroxylase (DBH), the enzyme responsible for the conversion of dopamine to noradrenaline. The latter approach is based on the premise that there is a concomitant release of DBH and noradrenaline from CA containing storage vesicles (Weinshilbaum, Thoa, Johnson, Kopin & Axelrod, 1971a). The interpretation of data from studies of this type is based on a knowledge that CAs in circulation may originate from the adrenal medulla and the axon terminals of sympathetic nerves and that DBH in circulation is thought to be of extra-adrenal origin (Weinshilbaum, Kvetnansky, Axelrod & Kopin, 1971b).

Recent data from studies with both human diabetics and drug-induced diabetic animals has pointed to the possibility of an abnormal activity of the sympathetic nervous system in this disease (Low, Walsh, Huang & McLeod, 1975; Head & Berkowitz, 1977). The experiments to be described in this communication are concerned with the quantitation of CAs and the activities of DBH that prevail in the circulation of untreated and diabetic rats. For the purpose of collecting blood we adopted the commonly employed method of decapitation followed by collection of blood from the trunk of the animals. By simultaneous measurement of plasma CAs and DBH activity we have attempted to determine to what extent adrenal-medullary discharge contributes to the CA content of blood collected in this manner.

**Methods** Sprague-Dawley rats (Charles River, Wilmington, Mass.) weighing 410 to 460 g and aged-matched diabetic rats (treated for 9 weeks before decapitation with the diabetogenic agent, Streptozotocin 65 mg/kg i.v.) weighing 103 to 295 g were used. The rats were fasted overnight, decapitated and the first 1.0 ml and a subsequent 5.0 ml of blood collected from the trunk of each animal. The procedures used for the collection of blood, the measurement of noradrenaline, adrenaline and dopamine were those described by DaPrada & Zürcher (1976). The radioenzymatic assay for CAs is based on the measurement of the 3-*O*-methyl-$^3$H derivatives of CAs resulting from incubation of test solutions containing CAs with S-adenosyl-L-methionine [methyl-$^3$H] (specific activity 6 to 10 Ci/mmol, Amersham) and partially purified rat liver catechol-*O*-methyltransferase. The activity of DBH in plasma was determined by the method of Nagatsu & Udenfriend (1972) with the modifications suggested by Kato, Kuzuya & Nagatsu (1974). Enzyme activity was expressed as nanomoles of octopamine formed per ml of plasma per minute.

**Results** The results of the CA analysis on plasma from control and diabetic rats are shown in Figure 1. The most striking feature of the findings was the large and significant ($P < 0.05$, unpaired Student's $t$ test) increase in adrenaline and noradrenaline that occurred in the second (5.0 ml) fraction of blood collected from the trunk of decapitated rats. In both fractions adrenaline was the predominant CA, and by way of contrast dopamine although present in all fractions, contributed minimally to the total CA contents. The above trends were evident for both control

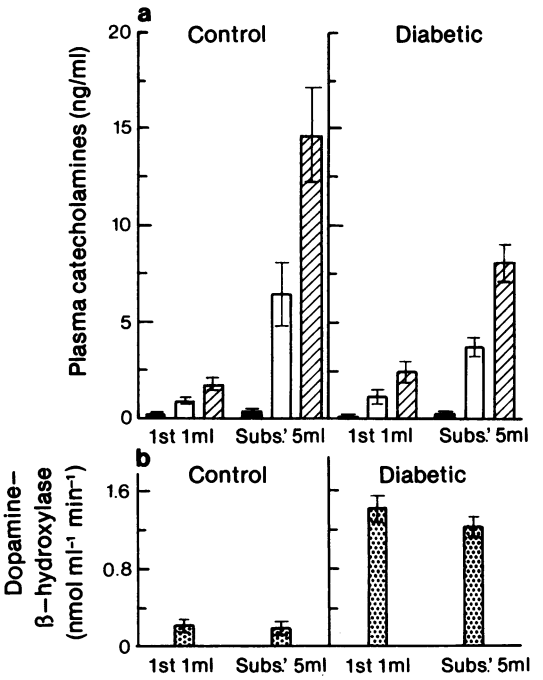


**Figure 1** Changes in circulating levels of (a) dopamine (solid columns) noradrenaline (open columns) and adrenaline (hatched columns) and (b) dopamine-β-hydroxylase activities (stippled columns) accompanying decapitation. Shown are the catecholamine contents and dopamine-β-hydroxylase activities in plasma obtained from the first (1.0 ml) and a subsequent 5.0 ml sample of blood collected from the trunk of decapitated diabetic ($n = 4$) and aged-matched control rats ($n = 4$). Vertical lines show s.e. means.

and diabetic rats. The levels of CAs in the first 1.0 ml of blood from diabetic and control rats were similar although the adrenaline concentrations in the 5.0 ml fraction of blood from diabetic rats were significantly ($P < 0.05$, unpaired Student's $t$ test) smaller than the corresponding control values.

DBH activity was detected in all samples and the activities present in the first (1.0 ml) and subsequent (5.0 ml) fractions were similar for each group of animals (Figure 1). Thus, the large increase in plasma CAs in later samples following decapitation was not matched by an increase in the activity of DBH. The activities of DBH in blood from diabetic rats was much greater (5 fold) than those seen for the controls. This increase applied to both the first 1.0 ml and subsequent 5.0 ml fractions of blood collected and was significant ($P < 0.05$, unpaired Student's $t$ test) for each comparison.

**Discussion** In light of evidence that adrenaline is localized primarily in the adrenal medulla and only a minimal component of the activity of DBH in blood, from decapitated rats, is of adrenal origin (Weinshilbaum *et al.*, 1971b) it is concluded that the major portion of the total CAs present in blood from decapitated rats is adrenal in origin. This conclusion follows from the observation that adrenaline and not DBH was dramatically elevated in the second fraction. These findings indicate that sampling of blood from decapitated rats is contra-indicated in studies where the circulating levels of CAs of extra-adrenal origin are sought. A similar conclusion was reported recently by Popper, Chuieh & Kopin (1977) based on the results of experiments in which the circulating levels of CAs were determined in rats subjected to varying degrees of stress.

The failure to see an increase in DBH activity accompanying decapitation is consistent with the concept that the adrenal glands are not the major source of DBH in blood from decapitated rats. However, in light of recent evidence this interpretation must be viewed with caution. An increase in circulating DBH and CAs attributed to adrenal medullary discharge accompanying haemorrhagic hypotension has been reported recently by Cubeddu, Santiago, Talmaclu & Pinardi (1977). It is noteworthy that in the latter study, the increase in DBH lagged considerably behind the rapid increase in circulating CAs. Cubeddu *et al.* (1977) suggested that this observation may be attributed to trapping of DBH in tissues after exocytotic release from the adrenal gland. It follows that the extremely short period of time between decapitation and collection of blood may account for the failure to see an increase in DBH in the present study and may well explain the failure of Weinshilbaum *et al.* (1971b) to demonstrate an adrenal origin of DBH in blood from decapitated rats.

The failure to observe elevated levels of CAs in the plasma obtained from diabetic rats is of interest in view of the large activities of DBH detected in the plasma from these animals. In light of the findings presented in this communication, there is a strong possibility that changes in circulating CAs released from extra-adrenal sites in the diabetic rat were masked by an adrenal medullary discharge of catecholamines accompanying decapitation. Studies are in progress using different methods of blood sampling which reflect more accurately the release of CAs from sites other than the adrenal medulla. In this way, it is hoped to determine whether the increase in DBH activity is related to an increase in the activity of the sympathetic nervous system in the diabetic rat. Based on the present findings alternative explanations for the elevated DBH activity such as changes in the kinetic properties of the enzyme or alterations in the plasma clearance rate of DBH must also be considered.

We gratefully acknowledge the technical assistance of Mr James Hempstead.

## References

CRYER, P.E. (1976). Isotope-derivative measurements of plasma norepinephrine and epinephrine in man. *Diabetes*, **25**, 1071–1085.

CUBBEDDU, L., SANTIAGO, E., TALMACLU, R. & PINARDI, G. (1977). Adrenal origin of the increase in plasma dopamine-β-hydroxylase and catecholamines induced by hemorrhagic hypotension in dogs. *J. Pharmac. exp. Ther.* **203**, 587–597.

DaPRADA, M. & ZÜRCHER (1976). Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the fentomole range. *Life Sci.*, **19**, 1161–1174.

HEAD, R.J. & BERKOWITZ, B.A. (1977). Altered indices of sympathetic nervous activity in diabetes. *Pharmacologist*, **19**, No. 2, 238.

KATO, T., KUZUYA, H. & NAGATSU, T. (1974). A simple and sensitive assay for dopamine-β-hydroxylase activity by dual wavelength spectrophotometry. *Biochem. Med.* **10**, 320–328.

LOW, P.A., WALSH, J.C., HUANG, C.Y. & McLEOD, J.G. (1975). The sympathetic nervous system in diabetic neuropathy. *Brain*, **98**, 341–356.

NAGATSU, T. & UDENFRIEND, S. (1972) Photometric assay of dopamine-β-hydroxylase activity in human blood. *Clin. Chem.* **18**, 980–983.

POPPER, C.W., CHIUEH, C.C. & KOPIN, I.J. (1977). Plasma catecholamine concentrations in unanesthetized rats during sleep, wakefulness, immobilization and after decapitation. *J. Pharmac. exp. Ther.*, **202**, 144–148.

WEINSHILBAUM, R.M., KVETNANSKY, R., AXELROD, J. & KOPIN, I.J. (1971b). Elevation of serum dopamine-β-hydroxylase activity with forced immobilization. *Nature, New Biol.*, **230**, 287–288.

WEINSHILBAUM, R.M., THOA, N.B., JOHNSON, D.G., KOPIN, I.J., & AXELROD, J. (1971a) Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. *Science*, **174**, 1349–1351.

*(Received May 3, 1978)*