Biochemical Pharmacology, Vol. 27, pp. 451–455. Pergamon Press, 1978. Printed in Great Britain.

METABOLISM OF [^{3}H]EPINEPHRINE IN RABBIT FETAL TISSUES*

KATHLEEN T. SHIVERICK and SAMUEL SOLOMON

Departments of Biochemistry, Experimental Medicine and Obstetrics and Gynecology, McGill University and the University Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada

(*Received* 25 *February* 1977; *accepted* 19 *May* 1977)

Abstract—[^{3}H]epinephrine was injected into rabbit fetuses through the uterine wall on day 26 of gestation and its metabolism was studied in fetal tissues. [^{14}C]metanephrine (MN) was synthesized using a soluble rat liver preparation and it, along with [^{14}C]epinephrine, was used as recovery markers for the quantitation of metabolites of [^{3}H]epinephrine. The radiochemical purity of MN, the predominant metabolite isolated from the fetal liver, was achieved by obtaining a constant [^{3}H]/[^{14}C] after sequential thin-layer chromatography. Thirty min after the injection of labeled hormone, [^{3}H]epinephrine comprised 25 per cent of the radioactivity in the serum and 30 per cent in the heart, while less than 5 per cent was found in the lung, liver, kidney and intestine. Unconjugated MN comprised 45 per cent of the [^{3}H] in the serum, 63 per cent in the heart and lung and 47, 46 and 40 per cent in the liver, kidney and intestine respectively. Conjugated MN is only found in liver, kidney and intestine and accounts for 35–40 per cent of the tissue radioactivity. Enzymatic hydrolysis of conjugates with β-glucuronidase in the presence and absence of sulfatase indicates that MN is present as a glucuronide conjugate. *O*-methylated, deaminated metabolites accounted for 27 per cent of the radioactivity in the serum, 28 per cent in the lung and between 7 and 16 per cent in the heart, liver, kidney and intestine. Thus, in the fetal rabbit, *O*-methylation is the predominant route of metabolism of injected [^{3}H]epinephrine, followed by extensive glucuronide conjugation in the fetal liver, kidney and intestine.

In a companion paper [1], we described the tissue distribution of [^{3}H] after the injection of [^{3}H]epinephrine in the rabbit fetus on day 26 of gestation. It was found in that study that the fetal liver, kidney and intestine accumulated larger amounts of radioactivity than the fetal heart. The data presented here were derived from experiments designed to determine in a quantitative manner the extent of [^{3}H]epinephrine metabolism in fetal tissues and the nature of the metabolites formed. The metabolism of catecholamines is mediated primarily by the enzymes monoamine oxidase (EC 1.4.3.4., MAO) and catechol-*O*-methyl transferase (EC 2.1.1.7., COMT) as well as conjugating enzyme systems [2]. MAO activity during the fetal and neonatal period is much lower than the levels found in tissue of the adult rat [3] and rabbit [4]. Ignarro and Shideman [5] demonstrated that the COMT activity in the chick embryo develops with a time course similar to the MAO activity. In the pre-viable human fetus, norepinephrine metabolism was shown to occur primarily through the action of COMT [6]. While sulfation of catecholamine metabolites has been reported in the human fetus [6], the activity of glucuronidating enzymes has been found to be generally low in fetal and neonatal tissues of human, guinea pig and mouse [7, 8]. In the companion paper mentioned above [1], we found that the *O*-methylated metabolite of injected [^{3}H]epinephrine in the rabbit fetus was conjugated in the liver but the nature of the conjugate was not determined.

* Supported by a Postdoctoral Training Fellowship of the American Lung Association (K. T. S.) and by grants from the U.S. Public Health Service (HDO-4365) and the Medical Research Council of Canada (MT-1658).

MATERIALS AND METHODS

Anion exchange resin AG1-X2 (200–400 mesh, acetate form) was purchased from BioRad Laboratories, Canada. β-Glucuronidase, B-10 and *S*-adenosyl methionine were obtained from Sigma Chemical Co., St. Louis, MS. and Ketodase from Warner Chilcott Co., Morris Plains, NJ. All other materials used in these investigations have been previously described [1].

Radioactivity was determined as described in the companion paper [1].

Radioactive compound. *dl*-Epinephrine[7-^{3}H]bitartrate (10 Ci/m-mole) and *dl*-epinephrine[7-^{14}C]bitartrate (48 mCi/m-mole) were purchased from New England Nuclear Corp., Canada. The purity of both labeled compounds was determined by thin-layer chromatography (t.l.c.) using cellulose MN 300 plates and the solvent systems *n*-butanol–glacial acetic acid–water (4:1:1) and isopropanol saturated with 1 N HCl. A single radioactive peak was observed in both systems and no labeled impurities were observed. [^{3}H]epinephrine was always used within 1 month of receipt. Non-radioactive standards were visualized on the plates with diazotized *p*-nitroaniline spray.

[^{14}C]metanephrine (MN) was prepared by enzymatic *O*-methylation of [^{14}C]epinephrine using COMT which was purified from rat liver according to the method of Coyle and Henry [9]. Methylation of [^{14}C]epinephrine was done by a modification of the method of Axelrod and Tomchick [10]. A mixture

of 2 μCi of [^{14}C]epinephrine, 40 μmoles magnesium chloride, 0.4 μmole *S*-adenosyl methionine, 200 μmoles of phosphate buffer, pH 7.8, and COMT (6.2 μg epinephrine methylated/ml/hr) were incubated at 37° for 60 min. The reaction was stopped with addition of 2.0 ml of 0.5 M sodium borate, pH 10.0. Then 100 μg of non-radioactive MN was added and the methylated compound was extracted with water-saturated ethyl acetate–methanol (10:1). The extraction was repeated twice and the combined organic phase was dried *in vacuo* at 37°. The residue was dissolved in ethyl acetate and purified by chromatography using sequential t.l.c. systems on cellulose MN 300 plates (0.3 mm thick). The first system used was isopropanol–15% ammonia (8:2). The peak of radioactive material having the mobility of MN was eluted and the resulting extract was chromatographed in the system *n*-butanol–ethanol–water (4:1:1) and again using the system *n*-butanol–acetic acid–water (4:1:1) in which the radioactive material migrated as a single band corresponding in mobility to MN. Purified [^{14}C]MN was eluted in 0.1 N acetic acid and used within 2 weeks as a recovery marker in metabolism studies. In Table 1 are shown the mobilities of the biosynthesized [^{14}C]MN as compared to standard MN in the systems employed.

Animals. The animals used in this study and the injection of [^{3}H]epinephrine and handling of tissues were described in the companion paper [1].

Metabolites. The methods used in the isolation and quantitation of [^{3}H]epinephrine and [^{3}H]metanephrine from tissue extracts were the same as described in the companion paper except that [^{14}C]-labeled epinephrine and [^{14}C]MN were used as recovery markers. The amount of [^{3}H] was first determined in the extracts of serum, heart, liver, lung, kidney and intestine, and the [^{14}C] compound was added so that a final [^{3}H]/[^{14}C] of approximately 10/1 was achieved. Radiochemical purity of the isolated metanephrine from the liver was established by obtaining a constant [^{3}H]/[^{14}C] ratio after chromatography on t.l.c. in the three different solvent systems described for the purification of the biosynthesized [^{14}C]MN (Table 1).

Conjugated epinephrine and MN were cleaved by enzymatic hydrolysis of an aliquot of the tissue extract prior to chromatography on alumina columns. Glusulase, a mixture of β-glucuronidase and sulfatase, was used in the initial studies. To an aliquot ($\frac{1}{3}$ to $\frac{1}{2}$) of the tissue extract were added an equal volume of 0.5 M sodium acetate buffer, pH 6.0, and 0.02 vol. Glusulase. The mixture was incubated at 37° for 24 hr and then applied directly onto alumina. β-Glucuronidase (Ketodase and Sigma B-10, 1000 units/ml sample) hydrolysis was done in the same buffer system. A comparison of Ketodase and Sigma B10 β-glucuronidase preparations showed no difference in the amount of conjugated metabolites released.

The *O*-methylated, deaminated metabolites of epinephrine, vanylmandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) were separated using the method of Caesar *et al.* [11] on the extract resulting from Glusulase hydrolysis. The effluent from the cation exchange resin was applied on a column (0.9 × 1.6 cm) containing 4 g of anion exchange resin AG1-X2 (200–400 mesh, acetate form). The column was eluted first with 25 ml water and then with 30 ml of 1.0 M ammonium acetate buffer, pH 4.8. When the water eluate was adjusted to pH 4 with 1 N HCl, extracted twice with 30 ml ethyl acetate and then chromatographed on t.l.c. in the system isopropanol—5% ammonia (8:2), a single labeled material was observed migrating with the mobility of MHPG standard. Likewise, when the ammonium acetate eluate was adjusted to pH 1 with 50% H_2SO_4, extracted with ethyl acetate and the extract chromatographed on t.l.c., a single radioactive peak was observed with an R_f of the VMA standard. The total recovery of [^{3}H] applied to the cation and anion exchange columns was 90–95 per cent.

RESULTS

Radiochemical purity of metanephrine isolated from the liver. In a previous study [1], we found that the predominant metabolite of [^{3}H]epinephrine in fetal rabbit liver had the characteristics of metanephrine. We therefore set out to establish the identity of this metabolite. Purified [^{14}C]MN was added to the perchloric acid extract of fetal liver to give a [3H]/[^{14}C] of approximately 10/1 (Table 2). The ammonium hydroxide (NH_4OH) eluate from the cation exchange resin was purified by sequential t.l.c. in three solvent systems in order to obtain a constant [^{3}H]/[^{14}C] ratio. Based upon the final ratio and the dis./min of [^{14}C]MN added, the total amount of [^{3}H]MN present in the extract of fetal liver was determined to be 85.7 ± 4.5 per cent (S. E. M.). These data are in agreement with the per cent of [^{3}H] in the original NH_4OH eluate containing MN, confirming the quantitative recovery of this metabolite.

Catecholamine metabolites in fetal tissues. The major metabolites isolated from the tissues taken 30 min after the injection of [^{3}H]epinephrine into the rabbit fetuses are shown in Table 3. Recovered

Table 1. Purification of biosynthesized [^{14}C]metanephrine (MN)*

Thin-layer chromatography system	Solvent mixtures	R_f	
		MN standard	[^{14}C]MN
A	Isopropanol–5% ammonia (8:2)	0.82	0.83
B	*n*-Butanol–ethanol–water (4:1:1)	0.38	0.37
C	*n*-Butanol–glacial acetic acid–water (4:1:1)	0.66	0.64

* [^{14}C]MN was prepared by enzymatic *O*-methylation of [^{14}C]epinephrine and purified by chromatography using sequential t.l.c. systems on cellulose MN 300 plates (0.3 mm thick). Non-radioactive MN standard was visualized with diazotized *p*-nitroaniline spray.

Table 2. Quantity and radiochemical purity of [^{3}H]metanephrine in fetal liver*

Procedures	Experiment No. 1	2	3	4	5
[^{3}H] in liver extract (dis./min × 10^{-3})	795	799	799	489	696
[^{14}C]MN added (dis./min × 10^{-3})	85	85	80	51	68
[^{3}H]/[^{14}C] in 3 N NH_4OH fraction	10.3	8.9	9.7	7.5	10.4
[^{3}H]/[^{14}C] after t.l.c. in system A	9.6	8.4	9.2	7.3	9.5
[^{3}H]/[^{14}C] after t.l.c. in system B	8.9	7.9	9.1	6.7	9.2
[^{3}H]/[^{14}C] after t.l.c. in system C	9.0	8.0	9.1	6.6	9.3

* [^{14}C]MN was added to extracts of fetal liver so that the [^{3}H]/[^{14}C] was approximately 10/1. The 3 N NH_4OH eluate from the cation exchange resin was purified by sequential chromatography using the t.l.c. solvent systems which are described in Table 1. The per cent of [^{3}H]MN present in the original fetal liver extract was calculated from the dis./min of [^{14}C]MN added and the final [^{3}H]/[^{14}C] obtained after chromatography in system C; the mean per cent ±S. E. M. of this calculation is 85.7 ± 4.5.

[^{3}H]epinephrine comprises 25 per cent of radioactivity in the serum and 30 per cent in the heart, while less than 5 per cent was found in the lung, liver, kidney and intestine. *O*-methylation is the predominant metabolic route in all the tissues shown. Unconjugated MN comprises 45 per cent of [^{3}H] in the serum, 63 per cent in the heart and lung and 47, 46 and 40 per cent of label in the extracts from liver, kidney and intestine respectively. By contrast, conjugated MN is only found in the liver, kidney and intestine, where it accounts for approximately 35–40 per cent of the radioactivity in the extracts from these tissues.

Oxidative deamination of methylated catecholamine is not a major route of metabolism in heart, liver, kidney and intestine, where 16 per cent or less of the [^{3}H] is present in this fraction. The comparatively large portion of methylated deaminated metabolites in the serum (27 per cent) may be due to metabolism by blood elements or may reflect poor retention within tissues where oxidative deamination is a more active process, as exemplified by the lung (28 per cent). In order to determine the nature of the *O*-methylated, deaminated metabolites, we attempted to separate VMA from MHPG in extracts of blood and lung after Glusulase hydrolysis. The lower line for blood and lung in Table 3 shows that these two metabolites are found in similar proportions in these tissue extracts.

Nature of conjugated metabolites. Tissue extracts from lung, liver, intestine, kidney and serum were separated into fractions containing conjugated and unconjugated metabolites. From data not shown here it was found that hydrolysis with the β-glucuronidase plus sulfatase preparation did not reveal the presence of conjugates of epinephrine in serum or any other tissue examined. While no MN conjugates were found in serum or lung, a significant amount of conjugated MN was present in liver, intestine and kidney (Table 3). Further characterization of the nature of the MN conjugate was attempted by comparing the results of differential hydrolysis with β-glucuronidase with and without sulfatase. The data in Table 4 indicate that MN is almost exclusively conjugated as a glucuronide in fetal liver, kidney and intestine.

Table 3. Metabolites of [^{3}H]epinephrine in fetal rabbit tissues*

Tissue extracts	Total dis./min	Per cent of total dis./min: Epinephrine	Metanephrine: Total	Free	conjugated		*O*-methylated, deaminated: Total	MHPG	VMA
Serum (6)	171,150 ± 18,800	25 ± 2	45 ± 2	45 ± 2	(6)	0	27 ± 2	12 ± 0.2	(5) 11 ± 0.2
Heart (4)	119,000 ± 11,200	30 ± 2	63 ± 3†				7 ± 0.5		
Lung (7)	229,480 ± 45,600	4 ± 0.3	68 ± 2	63	(3)	0	28 ± 2	7 ± 0.2	(5) 5 ± 0.4
Liver (5)	418,380 ± 21,400	1 ± 0.2	83 ± 1	47 ± 2	(5)	36 ± 2	16 ± 0.5		
Kidney (4)	234,060 ± 22,300	4 ± 0.2	88 ± 2	46	(3)	40	8 ± 0.3		
Intestine (5)	269,790 ± 15,600	5 ± 0.5	76 ± 2	40	(3)	35	16 ± 0.6		

* The amount of radioactivity in the metabolites shown is expressed as the per cent total dis./min in tissue extracts prepared from tissue obtained 30 min after injection of [^{3}H]epinephrine into fetal rabbits. Values shown are the means ± S. E. M. for the number of samples shown in parentheses, each involving pooled tissues from several fetuses. Conjugated MN was determined by the difference between MN content before and after hydrolysis with Glusulase.

† There was insufficient radioactivity in the extract of fetal heart to determine the amount of conjugated metabolites.

Table 4. Metanephrine conjugates in rabbit fetal tissues*

	Per cent of total dis./min as unconjugated metanephrine		
	Control	β-Glucuronidase plus sulfatase	β-Glucuronidase
Intestine	29.1	92.0	82.3
	44.9	73.5	68.4
	46.4	75.1	
Average	40.1	80.2	75.4
Kidney	40.9	87.5	86.8
	38.2	85.3	81.4
	59.5	83.4	81.9
Average	46.2	85.4	83.4
Liver	26.1	85.1	85.8
	40.7	86.7	82.8
	21.4	85.9	84.4
Average	29.4	85.9	84.3

* The amount of radioactivity present as unconjugated MN is expressed as the per cent of total dis./min in extracts of fetal rabbit tissues. Comparison is made between MN content before (control) and after the enzymatic hydrolysis of conjugated metabolites with β-glucuronidase alone (Ketodase) or in the presence of a sulfatase plus a β-glucuronidase (Glusulase).

DISCUSSION

The physiologic disposition of circulating catecholamines is initially dependent on membrane transport systems which mediate the intracellular uptake of the hormone. Such mechanisms are present in the axonal membrane of adrenergic neurons [12, 13] and in extraneuronal sites of various effector tissues [13–15]. Neuronal uptake and storage of norepinephrine have been established as an important mechanism for the inactivation of neurotransmitter [16, 17]. In contrast, the extraneuronal transport system appears to function as a mechanism for rapid inactivation of catecholamines released from the adrenal medulla into the circulation [18]. In peripheral tissues, COMT appears to be localized mainly in extraneuronal sites while MAO involved in catecholamine metabolism is considered to be largely inside adrenergic nerve terminals [19, 20]. The ontogenetic development of catecholamine uptake, storage and metabolism has been studied in relation to the development of the sympathetic nervous system [21]. In a previous study we found that the fetal rabbit heart during late gestation did not accumulate [^{3}H] in the 5-, 30- and 60-min intervals after injection of [^{3}H]epinephrine into the fetus [1]. These data suggested the absence of a functional capacity for catecholamine uptake in the fetal heart and were in agreement with histochemical studies which indicate that the rabbit heart does not have morphologically mature sympathetic nerve terminals until 1 week after birth [22].

Our present studies concerning the metabolic fate of [^{3}H]-epinephrine in a number of fetal tissues necessitated the use of sensitive assays for the separation and quantitation of very small amounts of metabolites. In order to accomplish this, [^{14}C]MN was biosynthesized (see Table 1) was used as a recovery marker along with [^{14}C]epinephrine. The radiochemical purity of the predominant metabolite in the liver, MN, was further established by purification on t.l.c. to a constant [^{3}H]/[^{14}C] ratio (see Table 2). Our present studies on catecholamine metabolism demonstrate that the fetal rabbit heart was the only tissue studied which contained significant levels of unmetabolized [^{3}H]epinephrine. While 63 per cent of the radioactivity in this tissue was present as MN, only 7 per cent was in the *O*-methylated, deaminated form, which suggests that the COMT activity is large compared to MAO activity (see Table 3). By contrast, Iversen *et al.* [21] demonstrated that the heart of the newborn rat exhibits a greater capacity to retain unmetabolized norepinephrine, was well as deaminated metabolites. Saarikoski [6] found a pattern of metabolites in the human fetal heart similar to that found for the newborn rat. Our data suggest that the fetal rabbit heart has a capacity for the retention of epinephrine, suggesting neuronal uptake, while the predominant metabolite, MN, is the product of the extraneuronal enzyme, COMT. Atwood and Kirshner [23] indicate that the progressive increase in the ability of the heart of the postnatal rat to accumulate norepinephrine is due to an increase in the number or storage capacity of synaptic vesicles.

In a previous study [1] we injected [^{3}H]epinephrine into fetal rabbits and found that the liver, kidney and intestine showed a significant uptake and retention of radioactivity over a 60-min period. Our present studies demonstrate that unmetabolized [^{3}H]epinephrine does not accumulate in any of these fetal tissues. Rather, *O*-methylation is the predominant metabolic route utilized because MN comprises 68 per cent of the labeled material in the lung, and 76–88 per cent in the liver, intestine and kidney. By contrast, Saarikoski [6] found significant accumulation of unmetabolized [^{3}H]norepinephrine in the lung, intestine and kidneys of the human midterm fetus, which most likely reflects the greatest maturity of the sympathetic nervous system [24, 25]. In the

intestine of the newborn rat, Iversen *et al.* [21] found that the extent of norepinephrine metabolism was comparable to that found in the adult. In studies on the ontogeny of axonal uptake of norepinephrine in the intestine of the fetal rabbit, Gershon and Thompson [26] demonstrated that the onset of specific uptake occurred between days 21 and 24 of gestation. Insofar as we found no unmetabolized [^{3}H]epinephrine in the intestine of the fetal rabbit, it appears that the developing adrenergic neuron on day 26 of gestation lacks retention or storage capacity for accumulated catecholamine.

The uptake of norepinephrine in the perfused lung of the adult rabbit has kinetic and pharmacologic characteristics common to both neuronal and extraneuronal transport in other tissues [27, 28]. In the lung of the fetal rabbit we found no accumulation of [^{3}H]epinephrine, while the presence of both *O*-methylated and deaminated metabolites reflects both COMT and MAO activity (see Table 3). The pattern of metabolites isolated is comparable to that found for the perfused adult rabbit lung [29]. Our failure in the previous study [1] to observe accumulation of [^{3}H] in the lung over 60 min may reflect a rapid efflux of metabolites from the tissue as suggested by Nicholas *et al.* [28].

While there is considerable variability in the activity of conjugating enzymes in different species and for different substrates before birth [7, 8, 30], our data clearly show a considerable capacity in the fetal rabbit for glucuronide conjugation as indicated by the presence of MN glucuronide in the liver, kidney and intestine (see Table 4). By contrast, these tissues in the rabbit, mouse, guinea pig and human fetuses exhibit low capacity for glucuronide formation for 5-hydroxytryptamine [30], *O*-aminophenol [7, 8] and steroids [31]. It is of interest that the rabbit fetal liver, intestine and kidney contain comparable amounts of conjugated and unconjugated MN. Dutton [7] and Stevenson and Dutton [8] found that for the fetal guinea pig, the stomach and kidney were quantitatively more important than the liver in glucuronide conjugation. By contrast, human fetal tissues exhibit a greater ability to conjugate catecholamines [6] and steroids [31] as sulfate esters. In these studies, no sulfate conjugates of catecholamine metabolites were found in rabbit tissues.

It can be concluded that epinephrine, exogenously administered to the rabbit fetus, is inactivated primarily by metabolism to *O*-methylated products. Assuming that COMT is primarily localized in extraneuronal sites in fetal tissues, as is the case for the adult [19, 20], our data suggest that extraneuronal uptake and metabolism constitute the predominant mechanism for disposal of circulating catecholamines in this species. These findings raise several questions relative to the hormonal regulation of catecholamine disposition via extraneuronal pathways. The classic inhibitors of extraneuronal uptake include estradiol, testosterone and corticosteroids [32]. More recent studies on the effects of fetal decapitation suggest that glucocorticoids present in the circulation of the rabbit fetus act to inhibit the antenatal development of MAO and COMT [33]. Kalsner [34] demonstrated that smooth muscle contraction is prolonged after inhibition of extraneuronal uptake of catecholamines by steroids as well as by inhibitors of COMT. This suggests the possibility that administration of various drugs during pregnancy, particularly glucocorticoids, may interfere with the mechanisms of catecholamine inactivation by the fetus, and may thereby potentiate the action of circulating hormone on fetal adrenergic receptors.

REFERENCES

1. K. T. Shiverick and S. Solomon, *Biochem. Pharmac.* **00**, 000 (0000).
2. I. J. Kopin, J. Axelrod and E. Gordon, *J. Pharmac. exp. Ther.* **236**, 2109 (1961).
3. N. Robinson, *J. Neurochem.* **14**, 1083 (1967).
4. J. Bojanek, K. Bozkowa and St. Kurzepa, *Biol. Neonate* **9**, 203 (1966).
5. L. J. Ignarro and F. E. Shideman, *J. Pharmac. exp. Ther.* **159**, 29 (1968).
6. S. Saarikoski, *Acta physiol. scand.* (suppl.) 421 (1974).
7. G. J. Dutton, *Biochem. J.* **71**, 141 (1959).
8. I. H. Stevenson and G. J. Dutton, *Biochem. J.* **82**, 330 (1962).
9. J. T. Coyle and D. Henry, *J. Neurochem.* **21**, 61 (1973).
10. J. Axelrod and R. Tomchick, *J. biol. Chem.* **233**, 702 (1958).
11. P. M. Ceasar, C. R. J. Ruthven and M. Sandler, *Br. J. Pharmac.* **36**, 70 (1969).
12. L. L. Iversen, *Br. J. Pharmac.* **41**, 571 (1971).
13. L. L. Iversen, *Br. med. Bull.* **29**, 130 (1973).
14. S. Z. Lightman and L. L. Iversen, *Br. J. Pharmac.* **37**, 638 (1969).
15. J. S. Gillespie, *Br. med. Bull.* **29**, 136 (1973).
16. S. Z. Langer, *J. Physiol., Lond.* **208**, 515 (1970).
17. J. Hughes, *Br. J. Pharmac.* **44**, 472 (1972).
18. L. L. Iversen, *Biochem. Pharmac.* **23**, 1927 (1974).
19. B. Jarott and L. L. Iversen, *J. Neurochem.* **18**, 1 (1971).
20. J. A. Levin, *J. Pharmac. exp. Ther.* **190**, 210 (1974).
21. L. L. Iversen, J. de Champlain, J. Glowinski and J. Axelrod, *J. Pharmac. exp. Ther.* **157**, 509 (1967).
22. W. F. Friedman, P. E. Pool, D. Jacobowitz, S. C. Seagren and E. Braunwald, *Circulation Res.* **23**, 25 (1968).
23. G. F. Atwood and N. Kirshner, *Devl. Biol.* **49**, 532 (1976).
24. R. E. Greenberg and J. Lind, *Pediatrics, Springfield* **27**, 904 (1961).
25. J. B. Read and G. Burnstock, *Devl. Biol.* **22**, 513 (1970).
26. M. D. Gershon and E. B. Thompson, *J. Physiol., Lond.* **234**, 257 (1973).
27. Y. Iwasawa and C. N. Gillis, *J. Pharmac. exp. Ther.* **188**, 386 (1974).
28. T. E. Nicholas, J. M. Strum, L. S. Angelo and A. F. Junod, *Circulation Res.* **35**, 670 (1974).
29. J. Hughes, C. N. Gillis and F. E. Bloom, *J. Pharmac. exp. Ther.* **169**, 237 (1969).
30. T. P. Rothman, L. R. Ross and M. D. Gershon, *Brain Res.* **115**, 437 (1976).
31. S. Solomon, C. E. Bird, W. Ling, M. Iwamiya and P. C. M. Young, *Recent Prog. Horm. Res.* **23**, 297 (1967).
32. L. L. Iversen and P. J. Salt, *Br. J. Pharmac.* **40**, 528 (1970).
33. H. Parvex and S. Parvez, *Biochim. biophys. Acta* **338**, 21 (1974).
34. S. Kalsner, *Br. J. Pharmac.* **53**, 267 (1975).