J. Pharm. Pharmacol. 1987, 39: 153–157

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REVIEW

Isomeric octopamines: their occurrence and functions

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p-Octopamine is a widely distributed invertebrate neurotransmitter (Evans 1978). It also occurs in the mammalian sympathetic nervous system, where its function is unknown (Axelrod & Saavedra 1977). Recent advances (Ibrahim et al 1984, 1985) in mass spectrometric analytical techniques have disclosed that, in addition to *p*-octopamine, *o*- and *m*-octopamine and the *N*-methyloctopamines (*m*- and *p*-synephrine) also occur naturally in mammals. Evidence indicates that *m*- and *p*-octopamine are located in sympathetic nerves with noradrenaline whereas *m*- and *p*-synephrine are found only in adrenal gland (Ibrahim et al 1985). The location of *o*-octopamine is unknown but it is probably not in the same anatomical structure as *m*- and *p*-octopamine and noradrenaline (Ibrahim & Williams 1985). This review summarizes the present knowledge concerning the distribution and possible functions of the three positionally isomeric octopamines.

DETECTION AND ASSAY

p-Octopamine occurs naturally in mammalian tissues in concentrations ranging from 6–60 pmol g^{-1}. These concentrations in normal animals could not be measured by two-dimensional paper chromatography, the only analytical technique available before 1969. In that year a radiochemical enzyme assay was introduced which could detect *p*-octopamine in concentrations as low as 0·3 pmol g^{-1} (Molinoff et al 1969). During the following decade that method was widely used for both the identification and quantitative analysis of *p*-octopamine in the vertebrate and invertebrate nerve systems (Axelrod & Saavedra 1977). Although it was known for some years that the enzyme used for the assay—phenylethanolamine *N*-methyltransferase (PNMT)—would also accept *m*-octopamine and *m*- and *p*-synephrines as substrates (Axelrod 1962), it was not until 1977 that *m*-octopamine was found in rat brain and salivary gland (Danielson et al 1977; Robertson et al 1977). This identification was achieved using a modified radiochemical enzyme assay with several additional thin layer chromatographic separations, and additional chemical processing, thereby increasing the complexity of the procedure. It was later established (Fuller et al 1981) that the enzyme PNMT could also accept *o*-octopamine and *o*-synephrine as substrates, although less efficiently than the corresponding *m*- and *p*-isomers. For those reasons, gas chromatography (GC)-mass spectrometry (MS) methods were sought for the unequivocal identification and quantitative determination of the three isomeric octopamines and the three corresponding synephrines. Electron impact GCMS methods using packed columns were developed in 1980, and successfully used to identify *m*- and *p*-synephrine in adrenal gland (Durden et al 1980; Midgley et al 1980). However, the presence of the other amines was not detectable by this method (i.e. 300–600 pmol g^{-1}). Recently, the introduction of capillary column GC and negative chemical ionization MS have improved the specificity and increased the sensitivity by 10–100-fold so that, in conjunction with deuterated internal standards, it is now possible to identify unequivocally and measure quantitatively the isomeric octopamines and synephrines in biological fluids (Ibrahim et al 1984) and tissues (Ibrahim et al 1985) in concentrations of 0·5–1 pmol g^{-1}.

OCCURRENCE

Five of the six isomers of octopamine and synephrine can be routinely measured in normal human urine (Ibrahim et al 1984) and the following concentrations (pmol mg^{-1} creatinine) were observed: *o*-octopamine 4, *m*-octopamine 14, *p*-octopamine 160, *m*-synephrine 11, *p*-synephrine 95. *m*-Octopamine coexists with *p*-octopamine in several sympathetically innervated organs of the rat (Ibrahim et al 1985)

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Table 1. Concentrations (pmol g^{-1}) of *m*- and *p*-octopamine in rat organs.

	meta-	*para-*
Adrenal	420	670
Heart	26	22
Spleen	25	25
Vas deferens	16	60
Brain	ND	5
Liver	7	7
Kidney	14	29
Large intestine	6	21
Bladder	ND	29
Lung	ND	13

ND, not detected (<1 pmol g^{-1}).

(Table 1), and it may be seen that there are similar quantities of each isomer in heart, spleen and liver. *m*-Octopamine occurs in lower concentrations than *p*-octopamine in adrenal glands, vas deferens, kidney and large intestine but could not be detected in brain, bladder or lung. Administration of a monoamine oxidase inhibitor caused a marked increase in the concentrations of both amines in every organ (Table 2)

Table 2. Concentrations (pmol g^{-1}) of *m*- and *p*-octopamine in rat organs after MAO inhibition.

	meta-	*para-*
Adrenal	1200	1800
Heart	780	1600
Spleen	720	1050
Vas deferens	720	2400
Brain	52	120
Liver	46	190
Kidney	130	270
Large intestine	150	300
Bladder	230	500
Lung	92	220

and administration of 6-hydroxydopamine, an agent causing chemical sympathectomy, effected complete loss of *m*- and *p*-octopamine from every organ except the adrenal glands. The occurrence of the isomeric synephrines is much less ubiquitous than the octopamines. The enzyme PNMT is highly localized in adrenal medulla with only traces found in other organs such as heart and brain (Axelrod 1962). The highest tissue concentrations of *m*- and *p*-octopamine are found in the adrenal medulla, consequently the occurrence of the corresponding synephrines (380 and 200 pmol g^{-1}, respectively) in this tissue was not unexpected. It is probable that *m*- and *p*-synephrine also occur in nerves containing PNMT in heart and brain, but in quantities too small to be detected by the present techniques. *o*-Synephrine has never been detected in any tissue or fluid and since *o*-octopamine is a poor substrate for PNMT (Fuller et al 1981) its absence is not surprising. The failure to detect *o*-octopamine in any organ (even after monoamine oxidase inhibition) suggests that it may occur in amounts below the lower detection limits of 0·5–1 pmol g^{-1}. *o*-Octopamine has been found in rat urine following monoamine oxidase inhibition (James et al 1983) and the major metabolite of *o*-octopamine, *o*-hydroxymandelic acid, is a natural constituent of both rat and human urine (Midgley et al 1979). We have recently detected *o*-octopamine in heart, vas deferens, spleen, kidney, lung and salivary gland of the rat after pretreatment with its metabolic precursor, *o*-tyramine, and a monoamine oxidase inhibitor. Chemical sympathectomy of the rat with 6-hydroxydopamine caused a significant drop (50%) in the daily excretion of the acid metabolites of *m*- and *p*-octopamine, noradrenaline and dopamine, but not that of *o*-octopamine (Ibrahim & Williams 1985). This was interpreted to mean that *o*-octopamine is localized in different tissues to those (presumably sympathetic nerve terminals) that contain *m*- and *p*-octopamine and the catecholamines.

TURNOVER

The localization and turnover of [^{3}H]noradrenaline and [^{3}H]*p*-octopamine have been investigated (Brandau & Axelrod 1972) and, although the concentration of *p*-octopamine in the rat heart was only about 1% that of noradrenaline, the half-life of *p*-octopamine (2·1 h) was significantly less than that of noradrenaline (13–15 h). It was concluded that *p*-octopamine constituted a substantial proportion of the total biogenic amines synthesized in adrenergic nerves. Turnover studies with *o*- and *m*-octopamine have not yet been performed but, if the daily excretion of the terminal urinary metabolites reflects the relative magnitude of biosynthesis of the corresponding biogenic amines (Table 3), it is apparent

Table 3. Concentrations (pmol g^{-1}) of amines in rat heart and total excretion (nmol day^{-1}) of acidic metabolites.

Amine	Concn (pmol g^{-1})	Acidic metabolite	Daily excretion (nmol day^{-1})
Noradrenaline	5900	Vanillylmandelic acid	20
p-Octopamine	22	*p*-Hydroxymandelic acid	480
m-Octopamine	26	*m*-Hydroxymandelic acid	6
o-Octopamine	<1	*o*-Hydroxymandelic acid	1

from these data that *p*-octopamine has a very much greater turnover than either of its isomers or noradrenaline. However, caution must be exercised in interpreting these results in the absence of data from experiments in which the disappearance from tissue of the radioactive compounds is measured, because the biogenic amines may or may not be taken up and stored in presynaptic vesicles after discharge by nerve stimulation, i.e. the discharge of a

biogenic amine from a nerve terminal may be followed by inactivation by enzymic conversion to a metabolite which is excreted, and/or by re-uptake and storage in presynaptic vesicles. In the latter case the excretion of the terminal metabolite would not accurately reflect turnover.

PHYSIOLOGY OF THE OCTOPAMINES

Because of their close structural relationship to noradrenaline and adrenaline, *m*- and *p*-octopamine and *m*- and *p*-synephrine were examined for adrenergic activity. These experiments, made in the early 1950s, were performed with racemates before recognition of the adrenoceptor subtypes α_1, α_2, β_1 and β_2. The racemic forms of *p*- and *m*-octopamine were found to be pressor agents in the dog but they were, respectively, 1/110 and 1/10 as active as (−)-noradrenaline (Lands & Grant 1952; Lands 1952) and the conclusion was drawn from the former data that the physiological actions of *p*-octopamine are similar to those of noradrenaline but that it is only 1/50 as active (Axelrod & Saavedra 1977). This appears to have been based on the reasonable assumption that only one enantiomer (implicitly we consider that this would be the (*R*) form, by analogy with noradrenaline) would be present naturally, and that the activity of the racemate would be one-half that of the endogenous bioactive form. Similar considerations lead to the conclusion that this enantiomer of *m*-octopamine would be 1/5 as potent as (−)-noradrenaline. However, when the (−)-*p*- (Kappe & Armstrong 1964) and (−)-*m*- (D'Amico et al 1956) isomers were eventually obtained, it was discovered that they were more active pressor agents (1/10 (Korol et al 1968) and 1/3 (Della Bella & Galli 1955), respectively) with respect to noradrenaline than predicted. The racemates of all three isomeric octopamines were tested for adrenergic activity in-vivo in the rat (Fregly et al 1979) and the relative pressor activities followed the order $m > p >> o$, although both *m*- and *p*-octopamine racemates were much less active than (−)-noradrenaline in the rat than in the dog. None of the three octopamines had any detectable β-adrenoceptor agonist activity in-vivo as measured by initiation of thirst and increase in tail skin temperature. An order of potency similar to that obtained by Fregly et al (1979) was also observed in rat aortic ring contractility in-vitro although in this test (±)-*m*-octopamine was almost as active as (−)-*m*-synephrine and (−)-noradrenaline (Ress et al 1980). Intracerebroventricularly administered (±)-*m*-octopamine, like (−)-*m*-synephrine, blocked angiotensin-II-induced water intake in the rat (Fregly et al 1984), an effect attributed to mediation by α_2-adrenoceptors. The (±)-*o*- and (±)-*p*-isomers were inactive in this respect.

Recently, we have resolved racemic *p*-synephrine and have modified previous methods (cf. D'Amico et al 1956; Kappe & Armstrong 1964) in order to obtain optically pure enantiomers of *m*- and *p*-octopamine. Currently the physiological activities of these isomers (which have been fully characterized), together with those of *m*-synephrine, are being measured on specific adrenoceptors (α_1, α_2, β_1 and β_2). Preliminary experiments on β_1-(chronotropic response of the guinea-pig isolated atria) and β_2-(relaxation of guinea-pig tracheal smooth muscle) receptors, indicate that all the (−)-isomers are partial agonists at both receptor types, their ranking of potency on β_1-adrenoceptors being noradrenaline > *m*-synephrine > *m*-octopamine = *p*-octopamine > *p*-synephrine, the most active isomer, (−)-*m*-synephrine, being approximately 1/100 as active as (−)-noradrenaline while both (−)-*m*- and (−)-*p*-octopamine were about 6000-fold less active than (−)-noradrenaline. Both (−)-*m*- and (−)-*p*-octopamine were more than four orders of magnitude less active than (−)-noradrenaline on β_2-adrenoceptors. These observations support our previous results with racemates and lead to the inevitable conclusion that the activities of (−)-*m*- and (−)-*p*-octopamine at β-adrenoceptors are too low for these compounds to have any physiological significance at those sites. Preliminary experiments with α_1-adrenoceptors in rat aorta gave results with the (−)-isomers that were also consistent with our earlier observations using racemates, (−)-*m*-octopamine being equiactive with (−)-*m*-synephrine in producing vascular smooth muscle contraction, both being about 6-fold less potent than (−)-noradrenaline, and (−)-*p*-octopamine and (−)-*p*-synephrine being about 1000-fold less active than (−)-noradrenaline. The potency ranking (−)-noradrenaline > (−)-*m*-octopamine = (−)-*m*-synephrine > (−)-*p*-octopamine = (−)-*p*-synephrine was also found with contractility of the rabbit saphenous vein (mediated predominantly by α_2-adrenoceptors) except that, at this receptor, the potency of the *m*-octopamine/*m*-synephrine pair is 150-fold less than that of noradrenaline. It is therefore probable that *m*- and *p*-octopamine are also partial agonists at α_1- and α_2-adrenoceptors and that their activity at those receptors is too low for them to perform a physiological function at those sites.

Possible physiological roles of the isomeric octopamines in vertebrates

Since *p*-octopamine exists naturally in amounts of less than 1% that of noradrenaline, and its physiological activity is only 1–2% that of noradrenaline, it has been argued that the compound is merely a metabolic by-product of noradrenaline synthesis and has no physiological function itself (Robertson 1981). The mere presence of a substance in the nervous system need not necessarily indicate an active function (Jones 1983), but it is equally evident that low concentrations of a substance need not obviate a function. The molar concentrations of the various octopamines are comparable with those of various neuropeptides present in the CNS and other organs (Krieger 1983) (Table 4). Moreover, low concentrations of endogenous amines or peptides may be offset by their high turnover rates and thus reflect the absence of a re-uptake storage mechanism rather than the absence of physiological function.

Table 4. Concentrations (mol mg^{-1}) of neurotransmitters.

Amino acids (brain)	10^{-6} to 10^{-8}
Acetylcholine, noradrenaline (brain)	10^{-9} to 10^{-10}
Peptides (brain)	10^{-12} to 10^{-15}
p-Octopamine (brain)	5×10^{-15}
p-Octopamine (heart)	2×10^{-14}
m-Octopamine (heart)	3×10^{-14}
o-Octopamine	$<10^{-15}$

Every treatment which changes the concentration of noradrenaline affects the concentration of *p*-octopamine in a similar, if not identical, manner. Thus exogenously administered [^{3}H]*p*-octopamine is taken up by sympathetic nerves of the cat spleen and released on stimulation of adrenergic nerves (Kopin et al 1964). This led to the proposal that *p*-octopamine functions as a co-transmitter with noradrenaline in the sympathetic nervous system and serves to modulate (reduce) the responses of adrenoceptors to noradrenaline (Axelrod & Saavedra 1977).

Since our discovery (Ibrahim et al 1985) that *m*-octopamine coexists with the *p*-isomer in the sympathetic nervous system, it is manifest that any treatment which affects the concentration of noradrenaline and *p*-octopamine will similarly affect that of *m*-octopamine. Moreover, it has recently been discovered that [^{3}H]*m*-octopamine is taken up in noradrenergic nerve terminals, accumulates in storage vesicles and is released together with noradrenaline upon stimulation (Reimann 1984). Consequently, the proposal of Axelrod & Saavedra (1977) that *p*-octopamine is a co-transmitter with noradrenaline must now be amended to include *m*-octopamine.

There are numerous examples of co-transmitters in all regions of the central and peripheral nervous systems in all species so far investigated, and it is probable that all neurons release more than one transmitter (O'Donohue et al 1985). Although most of the known examples of co-transmitter systems consist of two transmitters, several systems have been found to contain three co-transmitters. Multiple co-transmitters may be composed of peptides, non-peptides or a mixture of peptides and non-peptides. We suggest that the co-release of noradrenaline, *p*-octopamine and *m*-octopamine by mammalian sympathetic nerves may be an example of co-transmission by three non-peptides.

O'Donohue et al (1985) have also postulated several mechanisms by which co-transmitters could interact to produce neuromodulation: (i) both transmitters could bind to the same receptor, (ii) each transmitter could bind to a different receptor on the same cell, (iii) to different receptors on different cells or (iv) one co-transmitter could modify the action of a second co-transmitter. The low activity of *m*- and *p*-octopamine on α_1-, α_2-, β_1- and β_2-adrenoceptors would appear to obviate the first type of neuromodulation for those receptors.

If *m*- and *p*-octopamine do not bind physiologically to adrenoceptors, they may bind to specific octopamine receptors. Such receptors for *p*-octopamine are widespread in invertebrate nerve systems (David & Coulon 1985; Evans 1985) and have been subclassified pharmacologically in the locust (Evans 1981). However, experimental evidence for the existence of receptors specific for *p*-octopamine in the mammal is limited to a very few papers in which single neurons in spinal cord (Hicks & McLennan 1978a), thalamus (Dao & Walker 1980) and cerebral cortex (Hicks & McLennan 1978b) have been shown to respond differently to iontophoretically applied *p*-octopamine and noradrenaline. Additionally, *p*-octopamine has been shown to facilitate responses to iontophoretically applied noradrenaline on single neurons in rat cortex (Jones 1982) and cerebellum (Kostopoulos & Yarbough 1975). There is no evidence for the existence of receptors to *m*-octopamine to date.

CONCLUSIONS

Both *m*- and *p*-octopamine coexist with noradrenaline in mammalian sympathetic nerves and it seems likely that they are released as co-transmitters upon adrenergic nerve stimulation. The localization of

o-octopamine is unknown but indirect evidence suggests that it is not situated in the same structures which contain noradrenaline and *m*-octopamine. The mechanism by which *m*- and *p*-octopamine produce neuromodulation is unknown, although their low activities on α_1-, α_2-, β_1- and β_2-adrenoceptors indicate that any physiological function they may possess is not mediated via these sites.

Acknowledgement
The authors are indebted to Dr P. D. Evans, AFRC Unit, University of Cambridge, for helpful discussions.

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