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INDOMETHACIN-INDUCED INCREASE IN NORADRENALINE TURNOVER IN SOME RAT ORGANS

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1 The effect of a prostaglandin synthesis inhibitor, indomethacin, on noradrenaline turnover rate in various rat tissues was determined from the product of the endogenous noradrenaline concentration and of the rate constant of (−)-[^{3}H]-noradrenaline decline after injection of the labelled amine in tracer doses.

2 Treatment of the rats with indomethacin (5 mg/kg p.o. five times during 2.5 days) increased noradrenaline turnover rate 32-36% in submandibular gland, spleen and heart, and 4-17% in epididymal and subcutaneous adipose tissue.

3 The extrapolated [^{3}H]-noradrenaline content at time 0 was greater in heart and adipose tissue of indomethacin-treated animals than in controls, while it was not significantly changed in spleen and salivary gland.

4 There was no apparent relationship between tissue concentrations of intravenously injected [^{14}C]-indomethacin and the effect of indomethacin on noradrenaline uptake and turnover rate in the different tissues.

5 Indomethacin treatment did not affect monoamine oxidase and catechol *O*-methyltransferase activities in the different tissues.

6 The results are consistent with the hypothesis that indomethacin increases noradrenaline turnover in the rat by blockade of a locally operating feed back inhibition of transmitter release by prostaglandins. However, additional effects, such as an increased impulse traffic, cannot be ruled out.

Introduction

There is considerable *in vitro* evidence that prostaglandins modulate noradrenaline (NA) release from nerve endings in many tissues (Hedqvist, 1973). The possibility that this mechanism might be physiologically important has received circumstantial support by the finding, that prostaglandin synthesis inhibitors, including indomethacin, do cause an increased NA release from cat spleen (Hedqvist, Stjärne & Wennmalm, 1971), rabbit heart (Samuelsson & Wennmalm, 1971; Chanh, Junstad & Wennmalm, 1972), and guinea-pig vas deferens (Hedqvist, 1973; Fredholm & Hedqvist, 1973a).

The demonstration that indomethacin can increase urinary excretion of NA in rats (Stjärne, 1972; Junstad & Wennmalm, 1972) seems to provide the only indication that this mechanism might also operate *in vivo*.

We have examined this question more directly by determining the effect of indomethacin on NA turnover, i.e. the time dependent decrease in [^{3}H]-NA content in different tissues following intravenous (i.v.) injection of [^{3}H]-NA. The results indicate that indomethacin increases NA turnover in a variety of rat tissues. Furthermore our results suggest that increased NA release from the sympathetic neurones, rather than altered inactivation of NA released, is the main cause of this effect.

Methods

Materials

Male Sprague-Dawley rats (Anticimex strain) weighing 200-300 g were used throughout the study. (−)-[7-^{3}H]-Noradrenaline (9.2 Ci/mmol) was obtained from the Radiochemical Centre, Amersham. Both [2-^{14}C]-indomethacin and unlabelled indomethacin were kind gifts of MSD International. S-adenosyl-(−)-[methyl-^{14}C]-methionine (500 μCi/mmol and [ethylamine-^{14}C]-dopamine hydrochloride (16 μCi/μmol), used in the enzyme assays, were obtained from the Radiochemical Centre, Amersham. Unlabelled dopamine was obtained from Sigma (St Louis, U.S.A.). Other reagents and solvents were reagent grade from ordinary commercial sources.

Distribution of [^{14}C]-indomethacin

Six rats (200-260 g) kept at room temperature were given indomethacin, 5 mg/kg i.v. (in 0.5-0.8 ml 10% ethanol) containing 0.5 μCi[^{14}C]-indomethacin. After 2.5 h the animals were killed by decapitation and the organs specified below rapidly dissected out, blotted on filter paper and weighed. The organs were then homogenized in an Ultra-Turrax homogenizer in 7 ml ethanol. Following centrifugation a 6 ml aliquot of the ethanol was mixed with 12 ml toluene scintillator and the [^{14}C]-radioactivity counted. The results are presented as μg of indomethacin, based on the specific activity of indomethacin in the injected solution.

Indomethacin treatment

Indomethacin (5 mg/kg) was administered by means of a gastric tube twice daily for 2.5 days. Control animals received an equivalent amount of vehicle (1 ml/kg 1% ethanol). During the indomethacin treatment up to the time of death the animals were kept at 4° C.

Noradrenaline turnover

Two hours after the last dose of indomethacin or vehicle the animals were given (−)-[^{3}H]-NA (10 μCi) in the tail vein and were killed by a blow on the head after an additional time of 30 min to 24 hours. The heart, submandibular glands, spleen and subcutaneous and epididymal adipose tissue were then quickly dissected out, blotted dry and weighed. The organs were homogenized and centrifuged and the supernatant adsorbed on alumina. Noradrenaline in the eluates was assayed fluorimetrically (Euler & Lishájko, 1961) and radioactively measured in a liquid scintillation spectrometer, as described below.

Monoamine oxidase (MAO)-activity

MAO activity was determined according to Wurtman & Axelrod (1963). Tissues from indomethacin-treated and control animals of cold were homogenized in 10 volumes of cold isotonic KCl. After centrifugation at 4000 *g* for 30 min, 25 μl of the supernatant was taken for assay. [7-^{14}C]-Dopamine was used as the substrate. Results are expressed as nmol (of dopamine deaminated) (mg protein)$^{-1}$ 30 min^{-1}.

Catechol O-methyl transferase (COMT)-activity

COMT activity was determined essentially according to Giles & Miller (1967), using dopamine and S-adenosyl-(−)-[methyl-^{14}C]-methionine as substrates. The source of enzyme was 25 μl of the same extract as used for MAO determination. Incubations were performed at 37°C for 15 min after which time the reaction was stopped by 0.45 M borate buffer, pH 10.

Protein was determined according to Lowry, Rosenbrough, Farr & Randall (1951) in 1 N NaOH after precipitation with 0.1% deoxycholate and 10% TCA.

For the determination of radioactivity of non-aqueous samples 1-6 ml of sample was shaken with toluene containing 4 g/l of Omnifluor (New England Nuclear, Boston) whereas aqueous samples were shaken with Instagel (Packard Instr. Co) and counted in a Packard Tri-Carb liquid scintillation spectrometer. Corrections for counting efficiency were made by means of external standardization.

Results

Indomethacin treatment caused a marked reduction of body weight and of submandibular gland weight in animals kept at 4° C (Table 1). The same was true for animals kept at room temperature (not shown). The weight of subcutaneous adipose tissue of indomethacin-treated animals kept at 4° C was also lower than that of controls, but the difference may not be real since it is quite difficult to remove this tissue reproducibly.

Table 2 summarizes the results on NA-turnover in animals kept at 4° C. The assumption that endogenous NA content reflects a 'steady state' level was borne out by the finding that the NA content as measured 0.5, 4 or 24 h after the i.v. administration of labelled NA did not differ significantly in any of the rat tissues examined. As shown in the last column in the table the calculated rate of NA turnover in the tissues examined was increased by 4-36% in the indomethacin-treated animals. In heart and in the two types of adipose tissue this was due to a significantly increased fractional turnover rate constant. In the submandibular gland, on the other hand, this constant was significantly lowered coincident with a significant elevation of the 'steady state' NA content.

It is of interest to note that the extrapolated [^{3}H]-NA content at time 0 was higher in heart and adipose tissue from indomethacin-treated animals than in the same tissues from control animals (Table 2), while it was not significantly changed in spleen and salivary gland. In the salivary gland we found no significant differences between control and indomethacin-treated rats in

Table 1 The effect of indomethacin treatment (5 mg/kg p.o. five times during 2.5 days) on the weight of whole rats and selected organs.

	No of animals	*Body weight*	*Heart*	*Spleen*	*Submandibular gland*	*Epididymal adipose tissue*	*Subcutaneous adipose tissue*
Control	24	183 ± 5	0.86 ± 0.18	0.63 ± 0.12	0.48 ± 0.13	1.23 ± 0.29	1.22 ± 0.30
Indomethacin	21	160 ± 1	0.77 ± 0.13	0.66 ± 0.14	0.32 ± 0.12	1.21 ± 0.45	0.77 ± 0.26
		$P < 0.005$	NS	NS	$P < 0.005$	NS	$P < 0.001$

The initial weight of the animals was 200 g, and both control and indomethacin-treated animals were kept at +4° C during the period of treatment. Results are given in g ± s.e. mean. Statistical analysis according to Student's *t*-test for unpaired data.

Table 2 The effect of indomethacin treatment on tissue noradrenaline (NA) content (mean ±s.e. mean) and NA turnover in rats kept at +4° C.

Tissue	*Indomethacin*	*Number*	*Uptake** (10^3 $dmin^{-1}$ g^{-1})	*Endogenous NA†* (μg/g)	*Rate‡* $k(h^{-1})$	*Turnover§* (μg g^{-1} h^{-1})	*Change in turnover* (%)
Heart	–	20	459 ± 55	0.52 ± 0.06	0.084 ± 0.003	0.044	+36
	+	20	621 ± 42	0.54 ± 0.06	0.110 ± 0.036	0.060	
			$P < 0.01$		$P < 0.05$		
Spleen	–	20	100 ± 42	0.60 ± 0.04	0.047 ± 0.018	0.028	+35
	+	20	120 ± 36	0.56 ± 0.06	0.068 ± 0.018	0.038	
Submandibular gland	–	20	201 ± 52	1.40 ± 0.07	0.089 ± 0.017	0.124	+32
	+	20	244 ± 49	2.32 ± 0.32	0.071 ± 0.003	0.164	
				$P < 0.01$	$P < 0.05$		
Subcutaneous adipose tissue	–	18	19 ± 4	0.039 ± 0.0006	0.070 ± 0.016	0.0027	+17
	+	20	33 ± 5	0.034 ± 0.0034	0.094 ± 0.015	0.0032	
			$P < 0.01$		$P < 0.01$		
Epididymal adipose tissue	–	18	12 ± 2	0.025 ± 0.0022	0.061 ± 0.013	0.0015	+4
	+	20	22 ± 3	0.020 ± 0.0033	0.079 ± 0.013	0.0016	
			$P < 0.01$		$P < 0.01$		

* 'Uptake' is presented as the intercept of the decline in [^{3}H]-radioactivity with the ordinate. Significance values based on Student's *t*-test.

† A correction (1/0.72) was made for losses on the alumina column. Significance based on Student's *t*-test.

‡ Slope of decline curve (fractional turnover rate constant) by the method of least squares. Significance values based on analysis of covariance.

§ Calculated according to the formula: Turnover rate = kC_0, where k is the fractional turnover rate constant and C_0 is the steady state level of NA (Costa *et al.*, 1966).

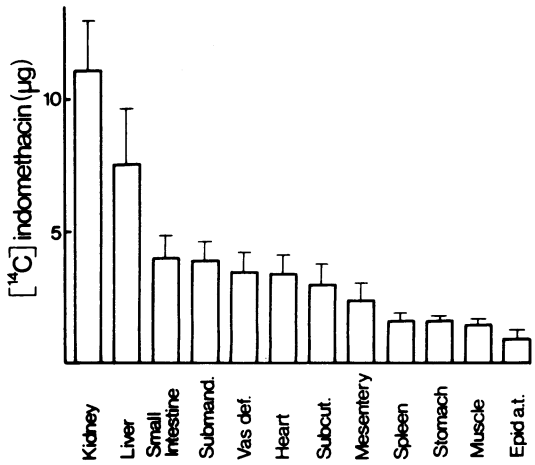

Figure 1 Distribution of indomethacin (μg) in rat tissues 2.5 h after i.v. administration of [^{14}C]-indomethacin (0.5 μCi/kg rat, specific activity 36 μCi/mmol). Mean values ± s.e. mean from six rats.

'uptake', whether this was expressed per unit weight of tissue or per μg of endogenous NA.

The data on [^{14}C]-indomethacin distribution between the tissues are presented in Figure 1. Our results are quite similar to those of Hucker, Zacchei, Cox, Brodie & Cantwell (1966) for those tissues that were examined in both studies. The plasma concentration was 13.2 μg/ml plasma 2.5 h after i.v. administration. There was no apparent relationship between the estimated tissue concentrations and the NA uptake level or turnover in the different tissues.

Indomethacin treatment lacked effect on MAO and COMT activities of liver, heart, spleen submandibular gland and epididymal adipose tissue (Tables 3 & 4). When COMT activity in adipose tissue extracts was assayed under the same conditions as those used for the other tissues the amount of [^{3}H]-methoxy-tyramine was too low to permit determination, in agreement with the results of Stock & Westermann (1963).

Table 3 MAO activities in heart, liver, spleen, submandibular gland and epididymal adipose tissue from control rats and rats treated with indomethacin (5 mg/kg five times during 2.5 days).

	MAO activity (nmol(mg protein)$^{-1}$ 30 min^{-1})	
	Control	*Indomethacin*
Heart	38.0	38.1
Liver	16.1	14.6
Spleen	4.9	4.7
Salivary gland	6.6	7.4
Epid. adipose tissue	12.9	12.2

Means of duplicate determinations in two animals in each group.

Discussion

The present results demonstrate that indomethacin treatment increased the rate constant for efflux of [^{3}H]-NA in all tissues studied, except for submandibular glands. Since the concentration of endogenous NA did not vary during the period in which efflux of [^{3}H]-NA was followed (up to 24 h), measurement of the turnover rate of NA from the steady state relationship in which the rates of formation and efflux of NA are equal is justified (Costa, Boullin, Hammer, Voger & Brodie, 1966). From this it is evident that indomethacin treatment increased NA turnover in all studied tissues. The reason why NA turnover was increased in submandibular glands despite a decreased fractional release of [^{3}H]-NA is in all probability the loss of weight which occurred during the period of indomethacin pretreatment. This caused a relative increase of endogenous NA in this tissue while the content of NA per gland was unchanged.

The data demonstrate a 30-40% increase in turnover rate in heart, spleen and submandibular

Table 4 COMT activities in heart, liver, spleen and submandibular gland from control rats and rats treated with indomethacin (5 mg/kg five times during 2.5 days).

	COMT activity (nmol(mg protein)$^{-1}$ 15 min^{-1})	
	Control	*Indomethacin*
Exp. 1		
Heart	0.5	0.7
Liver	47.9	52.2
Spleen	8.0	8.3
Salivary gland	3.8	5.3
Exp. 2		
Heart	0.5	0.6
Liver	37.4	38.8
Spleen	5.4	3.6
Salivary gland	6.4	5.8

Means of duplicate determinations in two animals in each group.

gland on the third day of indomethacin treatment. It is of interest that Stjärne (1972) found a similar increase in urinary NA excretion at that time. These results suggest that indomethacin causes a generalized increase in NA turnover.

The finding that the activities of the NA metabolizing enzymes were not affected by indomethacin tends to indicate that altered metabolic fate of the released noradrenaline is not a major cause of this increase in turnover. Clearly indomethacin might alter *in vivo* metabolism and the *in vitro* activity of enzymes differently. It is therefore interesting to note that Stjärne (1972) did not observe any change in the relative urinary excretion of (−)-[^{3}H]-NA as free intact amine by indomethacin treatment.

In the present experiments the extrapolated uptake of intravenously administered [^{3}H]-NA at time 0 was, if anything, increased by indomethacin. Volicer & Reid (1969) found that desipramine, an inhibitor of neuronal transmitter reuptake, actually decreased turnover. It is unlikely, therefore, that inhibition of the neuronal uptake mechanism could explain the observed effects with indomethacin. Since alterations in the disposition of NA is an unlikely explanation for the observed increase in turnover two main possibilities seem to remain: indomethacin causes either an increase in impulse flow or in the amount of NA released per impulse.

Considerable attention has been paid to the possibility that prostaglandins serve as physiological modulators of NA release (cf. Hedqvist, 1973). Aspirin-like drugs, such as indomethacin, which inhibit prostaglandin biosynthesis (Vane, 1971) might be used as tools to test this hypothesis. It should be pointed out in this context that homogenates of kidney from animals having received indomethacin according to the dosage schedule employed in this study converted only 3.2 ± 0.3% of 10 μg/ml [^{3}H]-arachidonic acid to prostaglandin E_2 compared to 7.0 ± 1.3% in controls (Fredholm, Hedqvist & Larsson, unpublished results). Thus there is evidence that indomethacin in this dose does inhibit prostaglandin biosynthesis. In a variety of tissues such as the cat spleen, rabbit heart and guinea-pig vas deferens, prostaglandins inhibit the release of overflow of NA per impulse and inhibitors of prostaglandin synthesis have the opposite effect (Hedqvist, 1973).

From these observations, it seems likely that indomethacin increases NA turnover in tissues and increases urinary excretion of NA by the removal of a prostaglandin mediated feed-back inhibition of NA-release per impulse.

Prostaglandin E_2 appears to have little effect on transmitter release (Fredholm & Hedqvist, 1973b) and indomethacin does not affect transmitter release during or after nerve stimulation in white adipose tissue (Fredholm & Hedqvist, 1975). Such an effect would require a prostaglandin concentration of the same order of magnitude as that required for inhibition of lipolysis.

Although controversial reports have appeared (Iliano & Cuatrecasas, 1971), the bulk of the recent evidence suggests that prostaglandins do not play a physiologically important role in the regulation of lipolysis in rat adipose tissue (Fain, Psychoyos, Czernik, Frost & Cash, 1973; Dalton & Hope, 1973; Fredholm & Hedqvist, 1974).

In the present study, NA turnover in adipose tissue was less affected by indomethacin than in the other tissues studied. This finding, which cannot be explained in terms of uneven distribution of indomethacin between the different tissues, is compatible with the view that this tissue is devoid of a physiologically operative prostaglandin mechanism.

Even though NA turnover was affected least in adipose tissue, it was slightly elevated after indomethacin treatment; it was not possible to test the significance of this finding statistically. However, the possibility that indomethacin also increases impulse traffic in adrenergic neurones must be borne in mind. Animals treated with indomethacin are hypoglycaemic (Fredholm & Hedqvist, 1975) and hypoglycaemia stimulates sympatho-adrenal activity (cf. Himms-Hagen, 1967). It is not known, however, whether this will add to the stimulation induced by low environmental temperature.

In fat cells isolated from rats pretreated with indomethacin NA diminished the lipolytic response (Fredholm & Hedqvist, 1975). It is therefore conceivable, albeit remotely, that indomethacin treatment might cause a reduction in receptor sensitivity which could lead to reflex increase in sympathetic nerve activity and increased NA turnover (Dontas & Nickerson, 1957; Dairman, Gordon, Spector, Sjoerdsma & Udenfriend, 1968).

In conclusion, the present data have provided evidence that indomethacin treatment increases NA-turnover in the rat. It is unlikely that this effect is due to an alteration in NA disposition. Our results are in harmony with the hypothesis that the effect of indomethacin is due to blockade of a locally operating feed-back inhibition of transmitter release by prostaglandins, although additional explanations such as an increased impulse traffic, cannot be ruled out.

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