trasting effects between systemic and local administration of PGEs may reflect a dose effect of PGEs on the vascular wall and edema formation.

Additional studies have demonstrated increased levels of PGE_2 in exudates at 3–6 hr after carrageenin administration [8, 18]. Although this later phase of edema formation is inhibited by aspirin and indomethacin [2, 3], this is only indirect evidence implicating PGE_2 as a pro-inflammatory agent. It is possible that the increased level of PGE_2 previously reported in acute carrageenin inflammatory sites is not the causative agent of the persistent edema. Several authors have shown that the so-called prostaglandin phase of vascular permeability change is dependent on the generation of superoxide anion (O_2^-) and is inhibited by superoxide dismutase and specific scavengers of oxygen-derived free radicals [19]. Since oxygen-derived free radicals and their metabolites have been shown to cause tissue injury, it is possible that their production is responsible for the delayed carrageenin-induced edema formation [20–22].

Recent studies have also shown that the effects of indomethacin and aspirin are not as specific as originally described. Since both compounds have been shown to inhibit the production of lipoxygenase products of arachidonic acid metabolism [23, 24], and indomethacin to inhibit oxygen free radical production [25] and release of lysosomal enzymes from inflammatory cells [25, 26], it is possible that the delayed permeability changes induced by carrageenin are not secondary to PGE_2, as previously described, but are the result of the local production of lipoxygenase products and/or oxygen-derived free radicals. The increased PGE levels demonstrated in early acute inflammatory reactions may, in fact, represent a modulating system that controls and limits the extent of the inflammatory response.

The data demonstrate that systemic treatment of rats with prostaglandins of the E series inhibited carrageenin-induced edema formation. In addition, not only were the acute changes in vascular permeability inhibited but the delayed persistent permeability change previously attributed to the local generation of prostaglandins was also suppressed. The data support the previously described anti-inflammatory effects of systemic treatment with prostaglandins of the E series *in vivo* and suggest that they may be of future use in therapeutic modulation of edema formation and inflammatory reactions.

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Methylation of norepinephrine and α-methylnorepinephrine in brain

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Because of the anatomic location of epinephrine-forming neurons in rat brain, attention has been focused recently on their possible role in the central regulation of blood pressure [1–3]. The possibility that some antihypertensive drugs act by influencing epinephrine-forming neurons or receptors for these neurons in brain has also been suggested [2–5].

Recently, Beart *et al.* [6, 7] have reported that α-meth-

yldopa reduced epinephrine concentration in rat hypothalamus. α-Methyldopamine and α-methylnorepinephrine are formed as metabolites from α-methyldopa, and especially α-methylnorepinephrine is thought to contribute importantly to the pharmacologic actions of α-methyldopa [8, 9]. Beart *et al.* [7] suggested that α-methylepinephrine might be formed in nervous tissue and referred to earlier claims [8] of identification of α-methylepinephrine in tissues high in activity of norepinephrine *N*-methyltransferase (EC 2.1.1.28, abbreviated PNMT for phenylethanolamine *N*-methyltransferase). Beart *et al.* [7] mentioned unpublished observations of their own that α-methylnorepinephrine is a substrate for hypothalamic PNMT *in vitro*.

Goldberg *et al.* [9] recently reported some pharmacologic effects of α-methylepinephrine. They were interested in this compound because of the presence of PNMT in brain stem nuclei where α-methyldopa is thought to act in lowering blood pressure, and they made the tacit assumption that α-methylnorepinephrine would be a substrate for PNMT, suggesting that α-methylepinephrine could also be an active metabolite of α-methyldopa in brain, "particularly in those areas which contain PNMT".

Several years ago we had observed that α-methylnorepinephrine was a very poor substrate for rabbit adrenal PNMT *in vitro* (R. W. Fuller and B. W. Roush, unpublished data), and these recent papers concerned with α-methylnorepinephrine as a substrate for brain PNMT caused us to re-examine this compound. The results described here suggest that α-methylnorepinephrine is *N*-methylated very poorly by rabbit adrenal PNMT and poorly if at all by rat brain PNMT, although it can be *N*-methylated by some other enzyme(s) in brain.

The activity of PNMT was measured radiometrically with *S*-adenosyl-L-methionine[methyl-^{14}C] (New England Nuclear Corp., Boston, MA) as the methyl donor and either (-)norepinephrine bitartrate (Winthrop Laboratories, New York, NY) or (±)erythro-α-methylnorepinephrine hydrochloride (Regis Chemical Co., Morton Grove, IL) as the methyl acceptor. The enzyme preparation was a partially purified ammonium sulfate fraction from the cytosol obtained by centrifugation of a homogenate of rat brain stem or rabbit adrenal glands [10, 11] (Fig. 1 and Table 1) or a crude homogenate of rat brain regions. With the adrenal enzyme, where PNMT activity is high and interfering enzymes are negligible, precipitation of unreacted *S*-adenosylmethionine by Reinecke salt followed by sampling the supernatant fraction after centrifugation was the method of enzyme assay [11]. With the brain enzyme, the radioactive catecholamine product was extracted by previously described procedures [12] based on the methods of Henry *et al.* [13].

Figure 1 compares the rates of methylation of norepinephrine and α-methylnorepinephrine by PNMT from rabbit adrenal glands and from rat brain stem. With the rabbit adrenal enzyme, the α-methyl compound was a poor substrate, eventually reaching a methylation velocity about one-fourth that for norepinephrine but only at a very high concentration (10,000 μM) compared to the optimum concentration of norepinephrine (40 μM). In brain stem, PNMT activity was much lower. Norepinephrine was methylated more rapidly than α-methylnorepinephrine at all concentrations tested, the calculated theoretical V_{max} for α-methylnorepinephrine being about half that for norepinephrine. Thus, α-methylnorepinephrine appeared somewhat better as a substrate in relation to norepinephrine with the brain enzyme that with the adrenal enzyme.

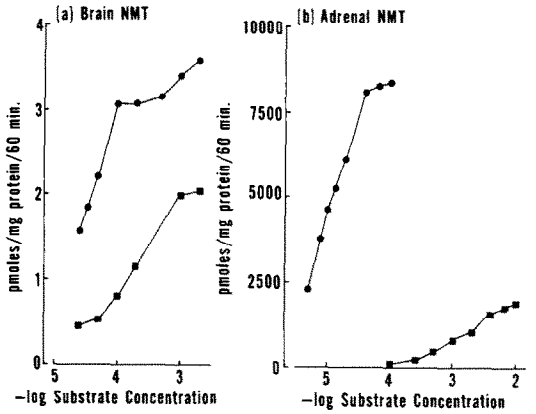

Fig. 1. Comparison of norepinephrine (●) and α-methylnorepinephrine (■) as substrates for PNMT preparations from (a) rat brain stem and from (b) rabbit adrenal glands.

To investigate whether the methylation of α-methylnorepinephrine by the brain enzyme preparation was actually due to PNMT, we examined the effect of a PNMT inhibitor. Table 1 shows that LY134046, a potent inhibitor of PNMT [14], prevented the methylation of norepinephrine by the brain enzyme but had no effect on the methylation of α-methylnorepinephrine. Table 1 also shows that *S*-adenosylhomocysteine (SAH), the product of transmethylation reactions involving SAMe as the methyl donor and an inhibitor of all transmethylases [15], inhibited the methylation both of norepinephrine and of α-methylnorepinephrine.

Figure 2 shows that hypothalamus and brain stem methylated norepinephrine to a much greater extent than α-methylnorepinephrine. These regions are known to be rich in PNMT [1, 16, 17]. In contrast, homogenates of cerebral hemispheres and cerebellum methylated norepinephrine to a much lesser extent, at approximately the same rate as the methylation of α-methylnorepinephrine. The rate of α-methylnorepinephrine methylation was similar in all four

Table 1. Inhibition by LY134046 and *S*-adenosylhomocysteine of the methylation of norepinephrine and α-methylnorepinephrine by rat brain stem *in vitro**

Inhibitor	Concentration (M)	Percent inhibition	
		Norepinephrine	α-Methyl norepinephrine
LY134046	3×10^{-6}	7	0
	1×10^{-5}	33	0
	3×10^{-5}	54	0
	1×10^{-4}	68	0
	3×10^{-4}	88	0
	1×10^{-3}	91	0
S-Adenosylhomocysteine	1×10^{-4}	94	90

* LY134046 is 8,9-dichloro-2,3,4,5-tetrahydro-1*H*-2-benzazepine hydrochloride. The substrates were present at 500 μM.

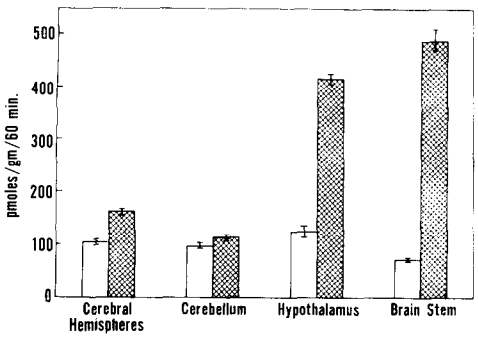

Fig. 2. Methylation of norepinephrine (cross-hatched bars) and α-methylnorepinephrine (open bars) by homogenates of rat brain regions. The substrates were present at 500 μM. Mean values ± standard errors for three rats are shown.

brain regions. These results suggest that the two substrates are not being methylated by the same enzyme, the α-methyl compound being acted on by a relatively ubiquitous enzyme whereas norepinephrine is methylated more rapidly by an enzyme localized in the hypothalamus and brain stem. Rat brain has been shown to contain "nonspecific" methyltransferase that can act on various arylalkylamines [18].

These studies lead us to suggest that α-methylnorepinephrine is a poor substrate for PNMT but that it can be *N*-methylated by another brain enzyme. Thus, the idea that α-methylepinephrine might be a metabolite formed in brain after administration of α-methyldopa is plausible, but the formation of α-methylepinephrine probably would not occur by PNMT action. There is then no basis for expecting that the formation of α-methylepinephrine would occur solely within PNMT-containing neurons or that these epinephrine-forming neurons would be preferentially affected.

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Evidence for a carbachol stimulated phosphatidylinositol effect in heart

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A wide variety of tissues show a selective increase in the incorporation of ^{32}P-inorganic phosphate into phosphatidic acid and phosphatidylinositol in response to certain neurotransmitters and hormones [1, 2]. Cholinergic agents such as acetylcholine and carbachol have been shown to induce this so-called phosphatidylinositol effect in pancreas [3], adrenal medulla [4], smooth muscle [5, 6], synaptosomes [7], avian salt gland [8] and parotid and lacrimal glands [9] by stimulating or acting on muscarinic receptors. Although various regions of cardiac tissue possess muscarinic receptors, evidence for stimulation of ^{32}P-incorporation into phosphatidylinositol and phosphatidic acid by cholinergic agents has not been reported previously. This study was undertaken to examine the effect of the non-