Substance P and neurotensin: Opposite effects on plasma cholesterol levels in ovariectomized conscious rats[1]

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Summary. Intravenous pulse injection of neurotensin produces a significant and dose-dependent increase in plasma cholesterol levels in ovariectomized conscious rats, whereas substance P has opposite effects. 4-Aminopyrazolo (3,4,-d) pyrimidine, (4-APP) also significantly lowers plasma cholesterol. The suppressive effects of both substance P and 4-APP were readily reversed by neurotensin, suggesting that the peptides act on hepatic lipoprotein secretion.

Neurotensin (NT) and substance P (SP) are 2 peptides which have been isolated and characterized in hypothalamic extracts[2,3] and which exhibit a wide variety of biological effects depending on the site and route of peptide administration[4-9]. None of the biological properties of NT and SP reported so far have been related to cholesterol, an obligatory precursor for steroid hormone production as well as membrane synthesis[10]. The present investigation sought to determine the effects of NT and its related peptide SP on circulating plasma cholesterol after their administration by i.v. pulse injections in ovariectomized rats bearing chronically implanted indwelling jugular venous cannulae[11,12]. 4-Aminopyrazolo (3,4,-d) pyrimidine (4-APP), a drug which selectively inhibits hepatic secretion of cholesterol[13] was also used to determine the possible site of action of the peptides in modifying circulating cholesterol levels. We report that i.v. NT produces a significant increase in plasma cholesterol while SP has an opposite effect, presumably by altering hepatic secretion of lipoproteins.

An indwelling silastic catheter was placed in the external jugular vein under light ether anesthesia[11,12]. Systemic i.v. pulse injections of freshly prepared NT and/or SP in 0.9% NaCl were made in a volume of 100 µl over a period of 60 sec[8]. 4-APP, 10 mg/kg in 10 mM phosphate buffer was given daily, i.p., for 3 days. Controls received an equal volume of vehicle. 1 group of rats was treated with synthetic LHRH as a peptide control. Blood samples were collected from the i.v. cannula at varying intervals while the animal was freely moving in the cage. Plasma cholesterol was estimated using the O-pthalaldehyde method[14].

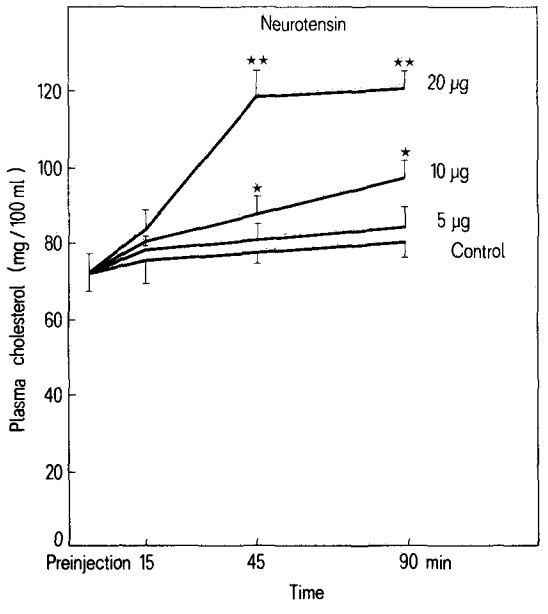

Figure 1. Plasma cholesterol levels after i.v. pulse injection of NT. Preinjection values for all groups are pooled and represented as mean ± SEM, (n = 6) as in figure 2. * $p < 0.05$; ** $p < 0.001$ vs preinjection level.

Chronic or acute treatment with 0.9% NaCl had no significant effects ($p > 0.05$) on plasma cholesterol levels. Injection of a 5 µg dose of NT did not alter plasma cholesterol levels, but they were significantly elevated by doses of 10 and 20 µg at 45 and 90 min in a dose-related manner (fig. 1). SP on the other hand significantly reduced the plasma cholesterol levels in a dose-related manner (fig. 2). Chronic treatment with SP or 4-APP also significantly lowered plasma cholesterol concentration. The suppressive effects of both SP and 4-APP were readily reversed by subsequent injection of a single dose of 20 µg NT (fig. 3).

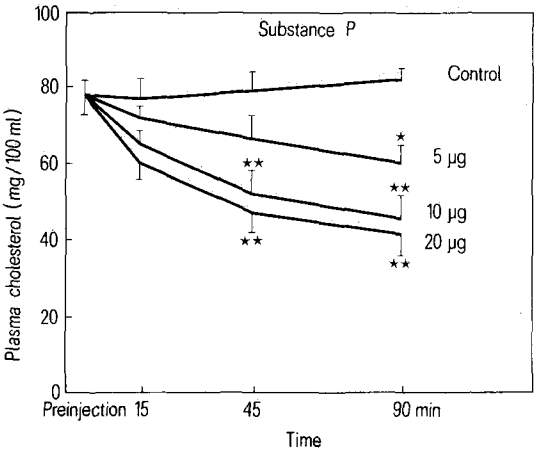

Figure 2. Plasma cholesterol levels after i.v. pulse injection of SP. p-Values as in figure 1.

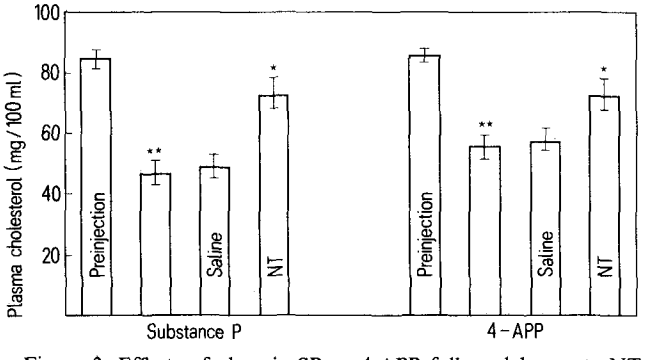

Figure 3. Effects of chronic SP or 4-APP followed by acute NT injection on circulating cholesterol. SP, 10 µg per rat or 4-APP, 10 mg/kg, in 0.5 ml 10 mM phosphate buffer was given daily for 3 days (n = 12 for each group). 24 h after the last injection a blood sample was drawn and immediately afterwards half the animals from each group received 20 µg NT and the other half received equal volumes of 0.9% NaCl. Blood samples were obtained 45 min after NT orsaline. The 1st and 2nd column of each group represent preinjection values and values after chronic treatment with SP or 4-APP, respectively. ** $p < 0.001$ vs preinjections value; * $p < 0.05$ vs SP or 4-APP + saline.

LHRH had no effect on circulating cholesterol levels (data not shown).

The present results demonstrate that NT and SP, 2 closely related peptides sharing certain properties, have opposite effects on circulating cholesterol levels. Several explanations may be proposed for the hyper- and hypocholesterolemic effect of NT and SP, respectively. Elevation of plasma cholesterol after i.v. NT suggests that the peptide may be involved at some step in the transport of cholesterol.

Considerable insight into the importance of blood cholesterol for regulating sterol metabolism in several tissues has come from studies using rats in which lipoproteins were reduced by 4-APP[15]. This model cannot, however, discern the mechanism(s) by which substance P mimics the action of 4-APP. Since NT antagonizes the effect of substance P and of 4-APP and 4-APP is known to selectively inhibit hepatic secretion of lipoproteins[15], we suggest that substance P, and also NT, may act on the liver.

A considerable concentration of NT and SP has been demonstrated in the small intestine of the rat[16], and some lipoproteins active in cholesterol transport synthesized in this segment of the gut are transported rapidly into the plasma via the mesenteric lymph[17,18]. Thus hypercholesterolemia may be induced by either intestinal overproduction or hypothalamic hypersecretion of NT followed by an increased delivery of NT to the gut or other tissues active in cholesterol transport. Determination of the physiological significance of these actions will require further studies, but the fact that both peptides are found in the brain and small intestine suggests that they may have some physiological role as humoral agents responsible for the occurrence of neurogenic hyper- and hypocholesterolemia. To our knowledge, these are the 1st results implicating substance P in the induction of hypocholesterolemia and in an antagonistic effect on NT-induced hypercholesterolemia. However, plasma levels of SP and NT have been reported to be far below the concentrations achieved by the injection of 5–20 µg peptide. It may be added that the doses used are rather high and bound to produce hypotensive effects. The precise point at which SP and NT act in producing these effects is difficult to identify. Since the liver is one of the sites of NT action[19], it is possible that NT acts to stimulate and SP acts to inhibit hepatic secretion of lipoproteins.

1 Supported by grants from the Indian Council of Medical Research (ICMR), New Delhi. K.R. was recipient of a UGC fellowship.
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Increase in pyridinoline cross-linking of mouse bone collagen induced by estrogen

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Summary. Pyridinoline, a non-reducible cross-link of collagen, was measured in the cortical bone of testectomized mice after repeated s.c. injections of estradiol benzoate. Pyridinoline content was increased significantly whereas the contents of hydroxylysine, and reducible cross-links remained unchanged.

A fluorescent cross-linking amino acid was recently isolated from collagen of ox tendon and bone and named pyridinoline[3]. Pyridinoline gradually increases with age in several tissues[4]. Aldimine cross-links, which are detectable after chemical reduction with $NaBH_4$ and are therefore called reducible cross-links decrease with age[5]. The decrease of solubility of collagen with age, therefore, is not explained by the stabilization of these cross-links in most species of animals, and they are considered as intermediates in the production of mature and stable cross-links[6,7]. Pyridinoline may possibly be one of the long-anticipated 'mature' cross-links of collagen, and 2 hypotheses are suggested about the pathway of its formation, which begin with hydroxylysine or its derivatives[8,9].

Steroid hormones, in particular estrogen, reportedly have various effects on connective tissue metabolism[10,11]. Estrogen affects the metabolism of connective tissues in vivo[12–15], and in vitro[16] suggesting that steroid hormones regulate the synthesizing process of fibrous proteins in fibrogenic cells. Moreover estrogen seems to accelerate extracellular maturation of collagen which was evidenced by decrease of solubility in skin collagen and an increase in the lysyl oxidase activity of collagen in bone, skin[17] and uterine cervix[18]. In the present experiment the effect of estrogen on the changes in the amount of cross-links of bone collagen, especially pyridinoline, was studied using mice.

Materials and methods. Male DDD mice[19] were fed a commercial diet and testectomized under ether anesthesia