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8 All gas chromatography employed a Varian model 2100 instrument equipped with a flame-ionization detector and a 1/8 inch ID X 6 foot glass column packed with 3% OV-1 on 100/120 Gas-chrom Q. Analytical runs were temperature-programmed from 60 to 260 °C at 10 °C/min using a carrier gas (N_2) flow rate of 30 ml/min. Retention times (min): *erythro*-3-hydroxyleucine TFA *n*-butyl ester, 6.7; TFA methyl ester, 5.1; 5,5-dichloroleucine TFA *n*-butyl ester, 10.1; TFA methyl ester, 8.6; 3-hydroxylysine TFA *n*-butyl ester, 11.6; TFA methyl ester, 10.1. Semipreparative work was performed under the same conditions using a 10:1 flow splitter and a carrier flow rate of 40 ml/min.
9 NMR data were recorded on a Bruker WM-300 FTNMR instrument. Chemical shifts are reported in ppm downfield from TMS. 3-Hydroxyleucine TFA methyl ester (**1**): 7.06 db, $J = 7.5$ Hz (NH); 5.04 dd, $J = 3.3$, 8.6 Hz (H-3); 4.97 dd, $J = 3.3$, 7.5 Hz (H-2); 3.84 s (3H, $COOCH_3$); 2.18 m, $J = 6.7$, 6.8, 8.6 Hz (H-4); 1.11 d, $J = 6.8$ Hz (CH_3); 0.97 d, $J = 6.7$ Hz (CH_3). 5,5-Dichloroleucine TFA methyl ester (**3**): 6.75 db, $J = 8.2$ Hz (NH): 5.77 d, $J = 2.9$ Hz (H-5); 4.70 ddd, $J = 3.1$, 4.0, 8.2 Hz (H-2); 3.81 s ($COOCH_3$); 2.15 m (H-4); 2.09 ddb, $J = 3.1$, 10 Hz (H-3a); 1.97 ddb, $J = 4.0$, 10 Hz (H-3b); 1.23 d, $J = 6.5$ Hz (CH_3). 3-Hydroxylysine TFA methyl ester (**6**): 6.96 db, $J = 8.8$ Hz (2-NH); 6.44 mb (6-NH); 5.62 m (H-3), 4.92 dd, $J = 2.2$, 8.8 Hz (H-2); 3.81 s (3H, $COOCH_3$); 3.42 m (H-6a, H-6b); 1.74 m (4H; 4-CH_2 and 5-CH_2).
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Moderate cooling depresses the accumulation and the release of newly synthesized catecholamines in isolated canine saphenous veins[1]

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Summary. Moderate cooling (from 37° to 24 °C) depressed the formation of ^{3}H-dopamine and ^{3}H-norepinephrine from ^{3}H-tyrosine by isolated canine saphenous veins. Cooling reduced the evoked release of newly synthesized catecholamine to the same extent as that of stored norepinephrine. Hence the augmentation by cold of the contractile response to sympathetic nerve stimulation observed in earlier work is not accompanied by an augmentated release of newly synthesized norepinephrine.
Key words. Cooling; canine saphenous vein; newly synthesized norepinephrine; release; electrical stimulation; accumulation.

In the isolated saphenous vein of the dog, moderate cooling augments the contractile responses to norepinephrine[18, 19]. This potentiation is due in part to an instantaneous increase in the affinity of postjunctional alpha-adrenoceptors[10]. Cooling augments the contractions evoked by sympathetic nerve stimulation more than those evoked by exogenous norepinephrine[10, 19], which suggests that the smooth muscle cells sense an increased concentration of the adrenergic transmitter in the junctional cleft. However, moderate cooling markedly decreases the stimulation-evoked overflow of both endogenous and ^{3}H-norepinephrine[9, 20]. Newly synthesized norepinephrine probably is stored in a small neuronal compartment and may contribute differently to the release process than the rest of the endogenous norepinephrine or than ^{3}H-norepinephrine recently taken up by the nerve endings[5, 8, 11–15]. Therefore, the aim of the present study was to investigate the effects of moderate cooling on the accumulation and release of newly synthesized catecholamines in canine saphenous veins.

Materials and methods

Both lateral saphenous veins were taken from mongrel dogs, anesthetized with sodium pentobarbital (30 mg/kg i.v.). The veins were placed in ice-cold modified Krebs-Ringer bicarbonate solution (mmolar composition: NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; glucose, 11.1; Ca EDTA, 0.026; control solution). They were cut into helical strips (5 mm in width) of 1 cm in length for incubation studies and 10 cm in length for superfusion studies.

1. Incubations

All tissues were equilibrated for 2 × 30 min in 2 ml of aerated (95% O_2-5% CO_2) control solution at 37 °C. They were then incubated for 30 min with 3,5-^{3}H-(−)-tyrosine (3.5×10^{-7} M; specific activity 53 Ci/mmole; New England Nuclear) either in 2 ml of control solution or in 2 ml of solution containing 50 mM K^+ (in equimolar re-

placement of Na^+). These incubations were carried out at either 24 °C or 37 °C. After the incubation, the tissues were washed with control solution and the radioactivity was then extracted with acetic acid[4]. A solution containing protectives and carriers (composition in M: HCl, 5; dopamine, 2.8×10^{-4}; norepinephrine, 4.2×10^{-5}; tyrosine, 5.5×10^{-5}; Na_2EDTA, 3.8×10^{-2}; sodium metabisulfite, 7.5×10^{-2}) was added to the samples which were then stored at −23 °C until chromatographic analysis.

2. Superfusion

2.1 Newly synthesized ^{3}H-catecholamines

Strips were incubated for 2 × 30 min in aerated control solution at 37 °C. The tissues were then incubated for 30 min with ^{3}H-tyrosine (3.5×10^{-7} M) in solution containing 50 mM K^+[4]. After incubation, the strips were suspended between two platinum electrodes in a chamber maintained at 37 °C, and continuously superfused with 3 ml/min of control solution. From the 30th to the 60th min of superfusion, the tissues were stimulated electrically (2 Hz, 9 V, 2 msec) by means of a stimulator (Grass S88). Samples of the superfusate were collected from the 20th to the 22nd, from the 28th to the 30th and from the 68th to the 70th min of superfusion (basal efflux) and from the 38th to the 40th, 48th to the 50th and 58th to the 60th min of superfusion (electrical stimulation). From the 40th to the 50 min of superfusion, the temperature was rapidly decreased from 37 °C to 24 °C.

All superfusate samples were collected in test tubes containing 2 ml of absolute ethanol and 0.6 ml of a solution containing carriers and protectives (composition in M: thioglycolic acid, 0.86; Na_2EDTA, 5.4×10^{-3} norepinephrine, 4.9×10^{-5}; tyrosine, 1.6×10^{-5}). The samples were then frozen at −23 °C until chromatographic analysis for determination of newly synthesized ^{3}H-catecholamines[4]. After superfusion, the tissues were blotted dry and weighed. Control tissues were studied in parallel at 37 °C throughout the experiment; the results obtained with cooling were corrected for the changes with time observed in these control veins.

2.2 ^{3}H-norepinephrine

Strips of the saphenous veins were incubated for 2 h at 37 °C in 5 ml of aerated control solution containing 7-^{3}H-(−)-norepinephrine (3×10^{-7} M; specific activity 18.8 Ci/mmole; New England Nuclear). After incubation, the strips were suspended for superfusion. A similar protocol was followed as described in 2.1. Samples of the superfusate were obtained from the 126th to the 130th and from the 166th to the 170th min of superfusion (basal efflux) and from the 136th to the 140th, 146th to the 150th, 156th to the 160th min of superfusion (electrical stimulation). The temperature was decreased from 37 °C to 24 °C from the 140th to the 150th min of superfusion. Superfusate samples were collected in test tubes containing carriers and protectives (composition in M: HCl, 5; sodium metabisulfite, 10^{-4}; Na_2EDTA, 5.4×10^{-5}; and 10^{-7} M of each of norepinephrine, 3,4-dihydroxyphenylglycol, 3,4-dihydroxymandelic acid, normetanephrine, 3-methoxy-4-hydroxyphenylglycol and 3-methoxy-4-hydroxymandelic acid). The samples were then frozen at −23 °C until column chromatographic separation of ^{3}H-norepinephrine from its metabolites[22,23]. The results obtained were corrected for changes with time as described previously[23].

3. Data analysis

For the newly synthesized ^{3}H-catecholamine determinations, the data were corrected for cross contaminations and recoveries as described previously[4]; the results are expressed as fmoles (10^{-15} moles) per g wet wt per 30 min of incubation or as fmoles per g wet wt per 2 min of superfusion. For ^{3}H-norepinephrine and its metabolites, the data are expressed as pmoles (10^{-12} moles) per 4 min of superfusion[24]. For statistical analysis, Student's t-test for paired or unpaired observations was used. p-values smaller than 0.05 were considered to be significant.

Results

1. Incubations

Four groups of saphenous vein strips were incubated with ^{3}H-tyrosine. Incubation in control solution (n = 6) at 37 °C yielded an equimolar tissue-accumulation of ^{3}H-norepinephrine and ^{3}H-dopamine (fig. 1). Cooling to 24 °C significantly reduced the tissue accumulation of both ^{3}H-catecholamines, the accumulation of ^{3}H-norepinephrine was decreased significantly more than that of ^{3}H-dopamine (fig. 1). Incubation in solution containing 50 mM K^+ (n = 4) at 37 °C significantly enhanced the tissue accumulation of both ^{3}H-norepinephrine and ^{3}H-dopamine (to 300 ± 68% and 252 ± 21% of the values noted in control solution, respectively); cooling to 24 °C decreased the tissue accumulation of ^{3}H-norepinephrine more than that of ^{3}H-dopamine (to respectively 18.5 ± 6.5% and 40.5 ± 9.9% of the values noted at 37 °C).

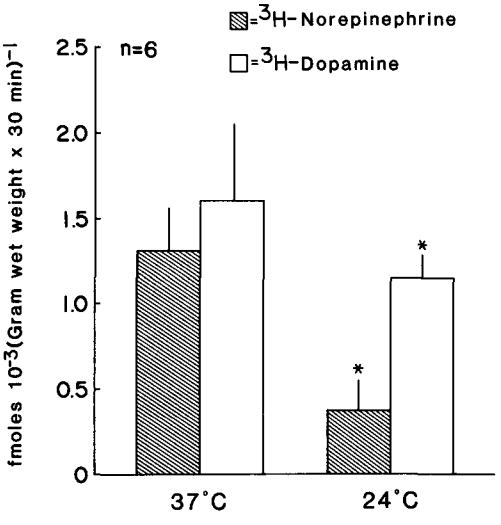

Figure 1. Effect of cooling on the accumulation of ^{3}H-norepinephrine (closed columns) and ^{3}H-dopamine (open columns) in six saphenous vein strips incubated in control solution with ^{3}H-tyrosine. The data are expressed in absolute values and shown as means ± SEM. * The effect of cooling is statistically significant ($p < 0.05$; Student's t-test for paired observations). ° The difference between the accumulation of ^{3}H-dopamine and ^{3}H-norepinephrine is statistically significant ($p < 0.05$; Student's t-test for paired observations).

2. Superfusions

2.1 Newly synthesized ^{3}H-catecholamines

In six strips, previously labeled with ^{3}H-tyrosine, electrical stimulation (2 Hz) caused an overflow of ^{3}H-catecholamines (from 2.8 ± 0.9 to 18.8 ± 4.0 fmoles/g/2 min). Cooling to 24 °C significantly decreased the stimulation-induced overflow of catecholamines (fig. 2).

2.2 ^{3}H-norepinephrine and its metabolites

In five veins previously incubated with ^{3}H-norepinephrine, electrical stimulation (2 Hz) evoked an overflow of intact ^{3}H-norepinephrine (from 48.4 ± 4.0 to 1578 ± 218 fmoles/g/4 min). Cooling to 24 °C significantly reduced the stimulation induced overflow of ^{3}H-norepinephrine (fig. 2). The decrease of the ^{3}H-norepinephrine overflow caused by cooling was not significantly different from that noted for the newly synthesized ^{3}H-catecholamines (fig. 2). The overflow of all ^{3}H-metabolites was also significantly reduced by cooling (DOPEG to 72 ± 4%, DOMA to 64 ± 2%, NMN to 42 ± 1%, VMA to 46 ± 2% and MOPEG to 67 ± 2% of the values obtained at 37 °C).

Discussion

The present experiments confirm earlier observations that isolated canine saphenous vein strips, incubated with ^{3}H-tyrosine, form and accumulate equal amounts of both ^{3}H-norepinephrine and ^{3}H-dopamine, and that the accumulation of both catecholamines can be enhanced if the incubation is carried out in the presence of an elevated K^+-concentration[4]. They demonstrate that moderate cooling markedly decreases the accumulation of ^{3}H-dopamine and ^{3}H-norepinephrine by the saphenous veins. Thus, a decrease in temperature inhibits the biosynthesis of the adrenergic transmitter, as it does for many processes in adrenergic nerve endings[21]. The present results do not allow to speculate whether cooling affects primarily the uptake of tyrosine, tyrosine hydroxylase or dopa-decarboxylase. The accumulation of ^{3}H-norepinephrine is reduced more by cooling than that of ^{3}H-dopamine independently of the K^+-concentration of the incubation solution. This difference could be due to an enhanced leakage or spontaneous release of norepinephrine, from the storage vesicles at 24 °C. This seems unlikely, since cooling decreases the basal efflux of ^{3}H-norepinephrine and its metabolites in the canine saphenous vein[9]. Alternatively, cooling may impair the vesicular uptake of ^{3}H-dopamine, and inhibit dopamine-β-hydroxylase. The rate of vesicular uptake is temperature-dependent[16]. A relative accumulation of dopamine in the neurones would be the likely consequence of blockade of vesicular uptake, in particular since at the lower temperature the activity of the intraneuronal monoamine oxidase is reduced[9]. A decrease in dopamine-β-hydroxylase activity by cold might have similar consequences.

The major goal of the present study was to determine the effect of cooling on the release of newly synthesized norepinephrine, under conditions where the release of ^{3}H-norepinephrine and total endogenous norepinephrine are reduced[9,20]. We measured the total release of newly formed ^{3}H-catecholamines (dopamine plus norepinephrine) since in the canine saphenous vein ^{3}H-norepinephrine constitutes more than 90% of the total[4]. At 37 °C, electrical stimulation evokes a marked overflow of newly synthesized catecholamines[4], which is reduced by cooling to 24 °C. Cooling has a comparable effect on the release of ^{3}H-norepinephrine previously taken up by the adrenergic nerve terminals; these results are similar to those obtained in earlier work[9,20]. Thus, moderate cooling (to 24 °C) decreases the stimulation-induced release of newly synthesized norepinephrine to the same extent as it decreases that of stored norepinephrine[9,20]. Hence, the greater effect of cooling on the contractile response to nerve stimulation than that to exogenous norepinephrine, cannot be explained by the effects of temperature on the exocytotic release of the neurotransmitter. It may be due either to the restriction of the movement of the neurotransmitter from the adrenergic neuroeffector junction caused by cooling[9], or by selective effect of changes in temperature on the postjunctional receptors facing the adrenergic varicosities[6,7].

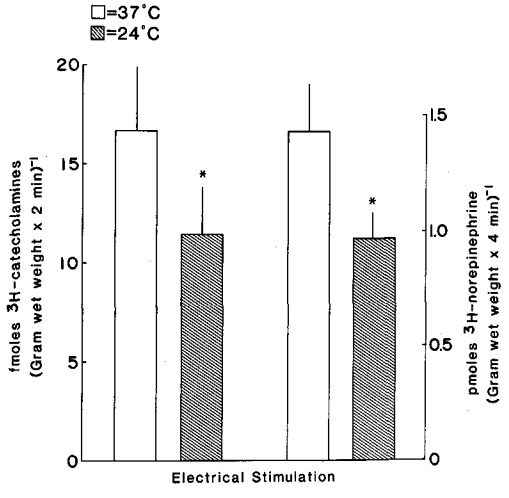

Figure 2. Effect of electrical stimulation at 37 °C (open columns) and at 24 °C (closed columns) on the evoked release of newly synthesized ^{3}H-catecholamines (left) and stored ^{3}H-norepinephrine (right) in canine saphenous veins. The data are expressed as absolute values (note the different scales) and shown as means ± SEM. * The effect of cooling to 24 °C is statistically significant ($p < 0.05$; Student's t-test for paired observations).

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Monoclonal antibodies against antigens on breast cancer cells

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F. Hoffmann-La Roche & Co Ltd, CH-4002 Basel (Switzerland), 30 April 1984

Summary. Of 360 mAb obtained in a cell fusion experiment with the spleen cells of a mouse immunized with a mixture of different human breast carcinoma cell lines, 30 mAb were selected which reacted more strongly with tumor cells than with (noncancerous) fibroblasts. These mAb were tested for reactivity with additional types of cancerous and noncancerous tissues. Two mAb showed high tumor selectivity, but the corresponding epitopes on individual tumor cells were heterogeneously expressed. The mAb will be evaluated for in vivo applications.
Key words. Murine monoclonal antibodies; tumor associated antigens; breast carcinomas.

Introduction

The advent of the mAb technology in 1975[5] renewed hopes of finding truly tumor specific antigens, bearing the promise of developing methods for tumor detection, localization and therapy. Despite efforts in many laboratories, however, antigens appearing exclusively on tumor cells have not yet been found. Early claims of tumor specificity, for example of mAb specific for carcinomas by recognition of an antigen named Ca[1,8], had to be abandoned after more extensive evaluation of the mAb reactivities with different types of normal human tissue[9]. Even if true tumor surface antigens were eventually to be found, they might not play a significant role in tumor localization and therapy, if the difficulties in finding them were a reflection of their low concentration. On the other hand, mAb reacting with malignant cells and restricted parts of normal tissue may be useful in spite of these normal tissue reactivities. In fact, such mAb have already proved useful, e.g. for radio localization of carcinomas of the colon[6] and of the breast[4]. Some of the unspecific background on such radioscintigraphs may be caused by normal cell reactivities of the mAb. The larger part of the background, however, is likely to be caused by unspecific effects, since significant background is also observed in nude mouse tumor models lacking normal tissue cross-reactivities. Furthermore, since such mAb usually do not react with all cells in any one tumor, mixtures (cocktails) of several mAb may have to be used. For therapy this may be further necessitated by the likelihood that even these antigens may be present at such low concentrations on cell surfaces that individual mAb cannot be effective[2]. Cocktails of mAb which react with restricted parts of normal tissue may furthermore have the advantage of