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Functional studies on human veins after storage at −190°C

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1 Human saphenous veins were immersed in foetal calf serum containing 1.8 M dimethylsulphoxide, slowly frozen to −70°C and stored in liquid nitrogen (−190°C).

2 Comparative *in vitro* studies on helical strips from unfrozen and frozen and thawed veins revealed that after thawing of frozen stored veins the contractile force development was unchanged, and the evidence suggested that the monoamine oxidase activity was unimpaired.

3 There was a good correlation between the pD_2 values of various 5-hydroxytryptamine receptor agonists and the blocking activities of various antagonists tested against 5-hydroxytryptamine (5-HT) and noradrenaline on unfrozen and frozen and thawed veins.

4 It is suggested that cryopreservation is a useful technique for storing human veins for pharmacological studies.

Introduction

Human vascular tissues for pharmacological studies can be obtained easily from surgery, but their availability is often unpredictable and irregular. When available, the supply generally provides more material than can be utilized at the time. Recently we published a simple and reliable method for the cryopreservation of canine saphenous veins and basilar arteries (Müller-Schweinitzer & Tapparelli, 1986). We now present data supporting the contention that frozen storage may also be considered an effective means of preserving human venous tissues for pharmacological studies.

Methods

Storage methods

Human saphenous veins, obtained from patients undergoing surgical removal of varicose veins, were placed in ice-cold Krebs-Henseleit solution (mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03) gassed with 95% O_2 and 5% CO_2, and transported to the laboratory within 4 h. Vein segments of about 15 to 20 mm length were distributed into 2 groups. Group 1 consisted of 'unfrozen veins' which were stored for 24 h in Krebs-Henseleit solution at +4°C before being used in organ bath studies. The veins of group 2, 'frozen and thawed veins', were placed in 2 ml liquid nitrogen storage ampoules (Gibco AG, Basel, Switzerland) filled with foetal calf serum (FCS) containing 1.8 M dimethylsulphoxide (DMSO) as a cryoprotective agent. The ampoules were placed in a styropor box (11 × 11 × 22 cm) and slowly frozen at a mean cooling rate of 0.6°C min^{-1} in a freezer maintained at −70°C. After 24 h the ampoules were transferred into liquid nitrogen (−190°C) where they were stored until use. Before being used the group 2 tissues were exposed for 30–60 min to −70°C before being thawed by placing the ampoules for 2.25 min in a 37°C water bath. Thereafter the vein segments were rinsed in a dish containing Krebs-Henseleit solution at 37°C and cut into helical strips for isometric recording as described previously (Müller-Schweinitzer, 1984; Victorzon *et al.*, 1986). Statistical analysis of data was performed using Student's *t* test.

Drugs

The following compounds were used: 5-hydroxytryptamine creatinine sulphate (5-HT), 5-methoxytryptamine hydrochloride (5-OCH_3-T, Fluka, Buchs, Switzerland), (−)-noradrenaline hydrogen tartrate (Hoechst, Frankfurt/Main, FRG), metitepin (Hoffmann-La Roche, Basel, Switzerland), spiperone (Janssen, Beerse, Belgium), cocaine hydrochloride (Lehner,

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Muttenz, Switzerland), tryptamine hydrochloride (T), yohimbine (Merck & Co., Darmstadt, FRG), dimethylsulphoxide (DMSO, Merck-Schuchardt, Hohenbrunn, FRG), prazosin hydrochloride (Pfizer, Karlsruhe, FRG), 5-methyltryptamine (5-CH_3-T), pargyline hydrochloride (Sigma, Munich, FRG), α-methyl-5-hydroxytryptamine creatinine sulphate (α-CH_3-5-HT, Upjohn, Kalamazoo, MI, USA), β-methyl-5-hydroxytryptamine hydrogen oxalate (β-CH_3-5-HT), ω-N-methyl-5-hydroxytryptamine oxalate (ω-N-CH_3-5-HT), N,N-dimethyl-5-hydroxytryptamine binoxalate (N,N-(CH_3)2-5-HT), 5-aminotryptamine oxalate (5-NH_2-T), 5-carboxamidotryptamine hydrogen maleinate (5-$CONH_2$-T), 4-hydroxytryptamine creatinine sulphate (4-HT), 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), pizotifen hydrogen malate and dihydroergotamine mesylate were synthesized by Sandoz Ltd, Basel, Switzerland. All compounds were dissolved just before use. Drug concentrations are given as molar concentrations throughout.

Results

Effect of DMSO on the contractile force

In preliminary experiments human saphenous veins were frozen in FCS containing DMSO concentrations ranging from 1.2 to 3.5 M. It was observed that the contractile force of strips from thawed veins which had been frozen in FCS containing DMSO concentrations below 1.5 M and above 2.5 M was considerably attenuated. Therefore in the present study human venous tissue was frozen in FCS containing 1.8 M DMSO. Under these conditions the maximum response to 5-HT of strips from frozen and thawed veins was 5.84 ± 0.76 mN (mean ± s.e.mean, $n = 70$) which was not significantly different from that produced by strips from unfrozen veins (5.05 ± 0.88 mN, $n = 70$). Furthermore, when the initial tension of 7.5 mN had been applied, strips from frozen and thawed veins relaxed within 30 min to a mean baseline tone of 4.06 ± 0.16 mN which again was not significantly different from that established by strips from unfrozen veins (3.95 ± 0.15 mN, $n = 15$) from the same patients.

Effects of agonists

On both unfrozen and frozen and thawed human saphenous veins, blockade of monoamine oxidase (MAO) activity with 30 μM pargyline (Vane, 1959) shifted the concentration-response curve for tryptamine to lower concentrations, thereby increasing the pD_2 value significantly by about 60 and 30 fold respectively. Therefore, the activities of all 5-HT-receptor agonists were investigated in the presence of 30 μM pargyline. Comparison of the pD_2 values of 12 5-HT-receptor agonists on human saphenous veins from both groups (Figure 1) revealed a good correlation ($r = 0.88$, $P < 0.001$) between the pD_2 values calculated on unfrozen veins and those determined on veins thawed after storage for 32 ± 2 days ($n = 15$) at −190°C. The pD_2 value for noradrenaline on strips from frozen and thawed veins (7.06 ± 0.07) was not significantly different from that determined on unfrozen veins from the same patients (6.95 ± 0.07, $n = 10$).

Effects of 5-HT receptor and α-adrenoceptor antagonists

Each antagonist tested against 5-HT or noradrenaline revealed similar inhibitory activity in frozen and thawed veins compared to that described previously (Müller-Schweinitzer, 1984; Victorzon *et al.*, 1986) for unfrozen veins. pA_2 or pD'_2 values for metitepin, yohimbine, spiperone, pizotifen and dihydroergotamine against 5-HT and for yohimbine, pizotifen and prazosin against noradrenaline are shown in Table 1. On both venous preparations the antagonists metitepin and yohimbine displaced the 5-HT curve to the right in a parallel fashion and dihydroergotamine, in addition to an agonist effect, produced a non-

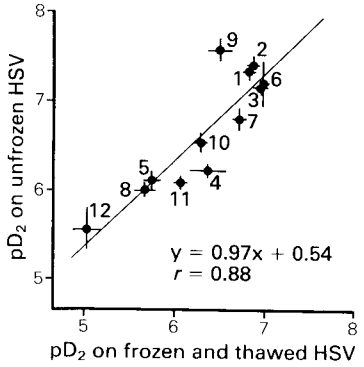

Figure 1 Correlation between pD_2 values of various 5-hydroxytryptamine (5-HT) receptor agonists determined on helical strips from human saphenous veins (HSV) thawed after storage for 32 ± 2 days at −190°C and those determined on unfrozen* preparations. The parameters were compared by linear regression analysis and the correlation coefficient (r) is given on the figure. The bars represent s.e.mean. *Data from Victorzon *et al.* (1986). 1 = 5-HT, 2 = 5-methyltryptamine, 3 = 5-methoxytryptamine, 4 = α-methyl 5-HT, 5 = β-methyl 5-HT, 6 = ω-N-methyl 5-HT, 7 = N,N-dimethyl 5-HT, 8 = 5-aminotryptamine, 9 = 5-carboxamidotryptamine, 10 = 4-HT, 11 = tryptamine and 12 = 8-hydroxy-2-(di-n-propylamino)tetralin.

Table 1 Antagonism of 5-hydroxytryptamine (5-HT) and noradrenaline on helical strips from 'unfrozen' and 'frozen and thawed' human saphenous veins (HSV)

	Unfrozen HSV	*Frozen/thawed HSV*
Antagonism of 5-HT		
Metitepin (pA_2)	8.20 ± 0.30 (6)	8.49 ± 0.23 (10)
Yohimbine (pA_2)	5.86 ± 0.08 (3)*	5.78 ± 0.14 (4)
Spiperone (pA_2)	8.41 ± 0.15 (12)†	8.26 ± 0.15 (9)
Pizotifen (pD'_2)	7.40 ± 0.11 (6)*	7.14 ± 0.19 (6)
Dihydroergotamine (pD'_2)	7.38 ± 0.12 (12)*	7.78 ± 0.19 (4)
Antagonism of noradrenaline		
Yohimbine (pA_2)	7.61 ± 0.13 (6)*	7.86 ± 0.15 (6)
Pizotifen (pA_2)	7.01 ± 0.23 (4)*	6.77 ± 0.06 (4)
Prazosin (max. inhibition)	−30% ± 2.10 (3)*	−24% ± 11.1 (3)

The pA_2 value for spiperone was determined as described previously (Victorzon *et al.*, 1986) at the 70% effect level of the control curve. Data are presented as means ± s.e.mean, number of determinations in parentheses. None of the parameters calculated on frozen and thawed veins is significantly different from the corresponding value determined on unfrozen veins.
*Data from Müller-Schweinitzer (1984); †data from Victorzon *et al.* (1986).

competitive depression of the maximal response to 5-HT. Furthermore, as observed with unfrozen veins (Müller-Schweinitzer, 1984; Victorzon *et al.*, 1986), both spiperone and pizotifen produced a biphasic shift of the 5-HT curve when tested on frozen and thawed veins. When tested against noradrenaline, again as observed on unfrozen veins, yohimbine and pizotifen displaced the noradrenaline curve to the right, while prazosin at 100 nM eliminated only about 25% of the maximal noradrenaline effect.

Discussion

The present experiments demonstrate that slow freezing to −70°C and subsequent storage in liquid nitrogen of human saphenous veins immersed in FCS containing 1.8 M DMSO yields an excellent cryopreservation of this tissue for pharmacological studies. Our experimental data present evidence that after thawing of frozen stored human veins not only the contractile force development but also the biochemical properties such as monoamine oxidase activity are unimpaired. Moreover, after cryopreservation the pD_2 values of various agonists and the blocking activities of various antagonists at both 5-HT receptors and α-adrenoceptors show a good correlation with previously published values on unfrozen veins (Müller-Schweinitzer, 1984; Victorzon *et al.*, 1986). Compared to our previous experiments, indicating a diminished constrictor response of frozen and thawed canine veins and arteries to 5-HT (Müller-Schweinitzer & Tapparelli, 1986), the present results suggest that freezing human saphenous veins by the same technique provides an even better cryopreservation than observed with canine blood vessels.

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