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Cryopreservation of Isolated Blood Vessels

By

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With 1 Table

Introduction

Organ bath studies with isolated blood vessels generally require freshly obtained vascular tissue, and usually the supply provides more material than can be used within the available time. Moreover, the availability of human vascular tissues, though it can be easily obtained from surgery, is often unpredictable and irregular. Thus the advantages of a simple, reliable storage method for ensuring the supply of adequate vascular preparations for pharmacological studies are readily apparent. We therefore investigated the pharmacological behaviour of canine and human saphenous veins after various freezing and storage methods.

Materials and Methods

Segments of freshly obtained canine veins (about 15–20 mm length) were placed either in Krebs-Henseleit solution (mmol/l: 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 5% CO_2 in oxygen, or immersed in 2 ml liquid nitrogen ampoules filled with fetal calf serum (FCS) containing various cryoprotective agents. Usually the ampoules were then placed in a polystyrol box (11 × 11 × 22 cm) and slowly frozen at a mean cooling rate of about 0.6 °C in a freezer maintained at —70 °C. After 4 to 24 h the ampoules were transferred into liquid nitrogen (—190 °C) where they were stored for several weeks. Before being used the tissues were exposed for 30 to 60 min to —70 °C and then thawed by placing the ampoules for 2.25 min in a 37 °C water bath. Thereafter the vessel segments were rinsed in a dish containing Krebs-Henseleit solution at 37 °C and cut into helical strips for isometric recording as described previously [1].

Drugs

The following agents were used: (–)-noradrenaline hydrogen tartrate (Hoechst, Frankfurt/Main, FRG), dimethyl sulfoxide (DMSO), N-methyl acetamide, polyethylene glycole 400 (PEG 400, Merck-Schuchardt, Hohenbrunn, FRG), glycerol, N-methyl formamide, dimethyl formamide, dimethyl acetamide (Fluka, Buchs, Switzerland). All compounds were dissolved just before use. Drug concentrations are given as molar concentrations. Statistical analysis of data was performed using Student's t-test.

Table 1
Percent maximal contractile response to noradrenaline of unfrozen and frozen/thawed canine saphenous vein strips.

Medium and protective agent		mol/l	Freezing			Storage		% Response		
			Speed	Temp.		Temp.	Time	mean	sem	n
K-H	no	–	–	–	–	–	–	100	± 8	(30)
K-H	no	–	–	–	–	+ 4°C	24 h	79	± 6	(30)
FCS	DMSO	1.8	slow	– 70°C	24 h	–190°C	4 w	59	± 4	(30)
FCS	DMSO	1.5	slow	– 70°C	24 h	–190°C	4 w	57	± 5	(6)
FCS	DMSO	1.8	slow	– 70°C	24 h	–190°C	3 w	51	± 5	(6)
FCS	DMSO	1.8	slow	– 70°C	–	– 70°C	4 w	42	± 1	(30)
FCS	DMSO	1.8	rapid	–190°C	–	–190°C	4 w	34	± 5	(6)
FCS	N-Me-ACETA	1.8	slow	– 70°C	24 h	–190°C	7 w	34	± 5	(6)
FCS	Di-Me-FORMA	1.8	slow	– 70°C	24 h	–190°C	4 w	33	±11	(6)
FCS	GLYCEROL	1.8	slow	– 70°C	24 h	–190°C	9 w	26	± 5	(6)
FCS	DMSO	2.6	slow	– 70°C	24 h	–190°C	9 w	19	± 5	(6)
FBS	DI-ME-ACETA	1.8	slow	– 70°C	24 h	–190°C	7 w	15	± 6	(6)
FCS	N-ME-FORMA	1.8	slow	– 70°C	24 h	–190°C	4 w	15	± 4	(6)
FCS	DMSO + PEG 400	1.5 + 0.5	slow	– 70°C	24 h	–190°C	4 w	14	± 7	(6)
FCS	DMSO	1.8	slow	– 20°C	24 h	–190°C	3 w	5	± 2	(6)
FCS	no	–	slow	– 70°C	24 h	–190°C	4 w	4	± 1	(6)
FCS	PEG 400	0.5	slow	– 70°C	24 h	–190°C	4 w	4	± 2	(6)
FCS	DMSO	1.8	slow	– 20°C	72 h	–190°C	3 w	0	± 0	(6)

K-H = Krebs-Henseleit solution; FCS = fetal calf serum: DMSO = dimethyl sulfoxide: PEG 400 = polyethylene glycol; N-Me-ACETA = N-methyl-acetamide; Di-Me-ACETA = di-methyl acetamide; N-Me-FORMA = N-methyl formamide: Di-Me FORMA = di-methyl formamide.

Results and Discussion

Preliminary Experiments. When living mammalian cells are frozen in physiological media the formation of ice crystals in intracellular and/or extracellular spaces, generally causes severe cell destructions and only few or even no cells survive. Cryoprotective agents such as dimethyl sulfoxide (DMSO) and many other compounds known to protect mammalian cells from damage during freezing and thawing, may reduce the amount of ice formed at any subzero temperature thereby increasing the recovery rate of frozen stored cells. However, different cell types differ in their requirements with regard to cooling rate, warming rate as well as with regard to the optimal cryoprotectant and its concentration. Consequently the presence of many different cell types within a tissue or organ implies that no one procedure can satisfy them all [4].

Therefore, preliminary experiments were performed to find a useful method of storing isolated blood vessels for pharmacological studies. The influence of various cryoprotective agents, storage temperatures and freezing and thawing methods on the contractile force of canine saphenous vein strips was determined as assessed by the maximal responses to noradrenaline (Tab. 1). Compared to the maximum contractile response to noradrenaline of unfrozen veins, storage in Krebs-Henseleit solution for 24 h at 4 °C diminished already the contractile force of the vein strips to about 80 %. Analysis of data obtained with frozen/thawed veins revealed that the best recovery, i.e., a contractile force of about 60 %, was obtained when veins had been immersed in FCS containing 1.5 or 1.8 mol/l DMSO as the cryoprotective agent, frozen slowly within 4 to 24 h to —70 °C and stored at —190 °C. The contractile force of veins was diminished to 50 % when the ampoules were kept for 72 h at —70 °C before being transferred into liquid nitrogen, and storage of the ampoules for 4 weeks at —70 °C resulted in a further reduction of the contractile force to about 40 %. Similarly, rapid freezing of the tissues by placing the ampoules directly into liquid nitrogen, diminished the contractile force of the veins to 35 %, supporting the suggestion that rapid freezing induces the formation of intracellular ice which may lead to cell damage [4]. Only negligible or even no recovery was obtained when veins were kept for 24 or 72 h at —20 °C before being transferred into liquid nitrogen. The same applies for veins which had been immersed in FCS containing 0.5 mol/l PEG 400 or in FCS without any protecting agent. The marked loss of recovery of veins immersed in FCS containing 1.5 mol/l DMSO plus 0.5 mol/l PEG 400 indicates that PEG 400 was toxic.

Validation Studies. Based on these data further validation studies were performed on canine basilar arteries and saphenous veins which had been frozen slowly to —70 °C and stored at —190 °C while being immersed in FCS containing 1.8 mol/l DMSO. Comparative pharmacological studies on unfrozen and frozen/thawed canine saphenous veins revealed that under these conditions after thawing the pD_2 values of both noradrenaline and 5-HT were slightly diminished while that of tryptamine was unchanged. Further experimental data suggested that main

biochemical properties such as monoamine oxidase activity, endogenous prostaglandin synthesis and neuronal uptake mechanisms are well preserved [1, 2].

The same method of cryopreservation has been applied to store samples of human saphenous veins. Functional studies on these tissues thawed after storage at —190 °C revealed that the contractile force development of frozen stored human veins was unchanged as compared to that produced by unfrozen preparations. Furthermore, there was a good correlation between the pD_2 values of various 5-HT receptor agonists on unfrozen and frozen/thawed veins and the same applies for the blocking activities of all antagonists tested up to now against 5-HT and noradrenaline on unfrozen and frozen/thawed human saphenous veins [2, 3].

In conclusion, slow freezing to —70 °C and storage in liquid nitrogen of canine and human venous tissue immersed in FCS containing 1.8 mol/l DMSO yielded an excellent cryopreservation of the main biochemical and functional properties and may be considered an effective mean of preserving and storing vascular tissues for pharmacological investigation.

Summary

Canine saphenous veins were immersed in fetal calf serum (FCS) containing various cryoprotective agents, slowly frozen and stored for several weeks at subzero temperatures. Pharmacological investigations of frozen/thawed tissues revealed considerable attenuation of the contractile force of frozen stored veins as compared to unfrozen veins. The best recovery after thawing of frozen stored canine veins was obtained on tissues which had been frozen slowly to —70° C and stored in liquid nitrogen while being immersed in FCS containing 1.8 mol/l dimethyl sulfoxide (DMSO). Though the maximum response to noradranline of helical strips prepared from these veins was diminished to about 60 % the evidence suggests that there may be a very good preservation of the main biochemical properties, such as monoamine oxidase activity, endogenous prostaglandin synthesis and neuronal uptake mechanism in veins stored under these conditions. The same method of cryopreservation was applied to store samples of human veins. Comparison of the pD_2 values for various agonists and of the blocking activities of various antagonists of both 5-HT receptors and alpha-adrenoceptors yielded an excellent correlation between the parameters determined on frozen/thawed and unfrozen human veins.

It is concluded that freezing isolated blood vessels may be considered an effective means of preserving and storing vascular tissues for pharmacological investigations.

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