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DOBUTAMINE RESPONSE OF ALCOHOLIC RAT HEARTS AFTER CERVICAL FRACTURE OR PENTOBARBITAL ANESTHESIA

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Our investigations of cardiac dysfunction after chronic alcohol consumption, in which we observed reduced hemodynamic and mechanical response to the β-adrenergic agonist, dobutamine, were performed using hearts isolated from rats anesthetized with sodium pentobarbital [1,2]. Sodium pentobarbital reportedly alters cardiovascular function in the intact animal [3,4], and recent work has suggested a persistent effect of the drug on the heart, specifically the inability of perfused hearts from pentobarbital-anesthetized rats to respond to increased extracellular calcium [5]. Since chronic alcohol consumption [2,6], sodium pentobarbital [7,8], and β-adrenergic agonists [9] all appear to affect cellular calcium flux, we were concerned about possible interactions of sodium pentobarbital anesthesia and alcohol-induced membrane alterations on the inotropic response to β-adrenergic agonists. In the present experiments, we studied the hemodynamic response to dobutamine of isolated working hearts from alcoholic and control rats killed after either pentobarbital anesthesia or cervical fracture.

Male Long-Evans hooded rats were fed an alcoholic or control liquid diet [2] for 57 to 61 weeks. The alcoholic diet supplied 39.2% of calories as ethyl alcohol. Animals were killed approx. 20 to 28 hr after their last meal period. Hearts were removed from heparinized rats (1000 Units/kg, i.p.) using one of the following procedures. Anesthetized animals (sodium pentobarbital, 65 ± 2 mg/kg, i.p.) were ventilated with room air while the heart was removed. Unanesthetized animals were killed by cervical fracture (Cervical Dislocators, Inc., Wausau, Wisconsin) prior to heart excision. The isolated working whole heart normoxia perfusion technique was as previously described [1,2,10,11]. Hearts were electrically stimulated at a frequency of 324/min. A ventricular function curve and response to 0.1 μM dobutamine were determined for each heart [1,2]. Four groups of rats were studied: controls killed after pentobarbital anesthesia (n = 6); alcoholics killed after pentobarbital anesthesia (n = 7); controls killed by cervical fracture (n = 7); alcoholics killed by cervical fracture (n = 7). Data were analyzed using Student's two-tailed t test for non-paired data [12] with $P < 0.05$ chosen as the level of significance.

Hearts from the pentobarbital-anesthetized alcoholic rats exhibited a significantly diminished inotropic response to dobutamine compared with pentobarbital-anesthetized controls, as we have previously observed [1,2]. The dobutamine-induced increases (absolute and/or percentage) in the peak relaxation rate ($-dP/dt_{max}$), aortic flow, stroke work, power (Table 1) and efficiency (not shown) were significantly less in the alcoholics killed after pentobarbital anesthesia. Unexpectedly, however, this difference in dobutamine response between control and alcoholic rat hearts was not evident when cervical fracture was used (Table 1). With that method of euthanasia, the response of the alcoholic rat hearts to dobutamine was comparable with that of their control group in all functional indices except the absolute response of coronary flow to dobutamine, which was significantly higher in the alcoholics than in the controls. Statistical analysis revealed that the dobutamine responses of the two control groups differed significantly, but the responses of the two alcoholic groups were similar (Table 1).

These results suggest that either (a) chronic ethanol consumption has no effect on cardiac β-adrenergic sensitivity, in which case the augmented response of pentobarbital-anesthetized controls relative to cervically-fractured controls is anomalous; or (b) chronic ethanol consumption reduces cardiac β-adrenergic sensitivity, in which case the diminished response of cervically-fractured controls, relative to pentobarbital-anesthetized controls, is deviant. Several considerations favor the latter possibility.

Firstly, β-adrenoceptor subsensitivity due to chronic alcoholism has been reported in brain and liver [13,14] possibly as a result of increased norepinephrine release or turnover induced by the ethanol metabolite, acetaldehyde [15,16]. Prolonged assault of the heart by catecholamines may be involved in production of the cardiac hypertrophy associated with alcoholism [17,18]. Other workers have demonstrated chronic catecholamine-induced β-adrenoceptor desensitization [19], notably in catecholamine-induced cardiac hypertrophy [20]. Thus, available evidence supports an hypothesis of catecholamine-induced cardiac β-adrenoceptor subsensitivity resulting from increased norepinephrine turnover or release caused by chronic alcoholism.

The diminished dobutamine response of cervically-fractured control rat hearts compared with pentobarbital-anesthetized controls may also be explained by a β-adrenoceptor subsensitivity or refractoriness acutely induced by a surge of catecholamines associated with the cervical fracture procedure. This possibility is supported by the report of significant changes in blood chemistry consistent with elevated catecholamine release that were detected in rats stunned by blows to the neck but not in rats anesthetized with pentobarbital [21]. Reduced β-adrenergic responsiveness of various tissues has been reported after a single exposure to catecholamine [22-25], although subsensitivity after very short time periods (e.g. several minutes) of exposure to elevated catecholamine levels has not yet been reported to our knowledge. The lack of effect of cervical fracture on the dobutamine response of the alcoholics, as indicated by the similarity of dobutamine response of the cervically-fractured alcoholics with the pentobarbital-anesthetized alcoholics, may be the result of a decreased potential of the alcoholics for acute catecholamine-induced desensitization, since they had already been chronically desensitized.

These preliminary data suggest that there is an effect of cervical fracture on subsequent response of the rat heart to β-adrenoceptor stimulation. Effects of pentobarbital anesthesia on the inotropic β-adrenergic response and/or Ca^{2+} flux alterations are also possible but cannot be assessed from the present data. Experiments utilizing reserpinized animals and animals anesthetized with pentobarbital or another agent followed by catecholamine infusion might be useful in confirming the mechanism we have suggested.

Table 1. Response of control and alcoholic rat hearts to dobutamine*

Group	Method of Euthanasia	$-dP/dt_{max}$		Aortic Flow		Stroke Work		Peak Power	
		Δ + DB†	%Δ + DB	Δ + DB	%Δ + DB	Δ + DB	%Δ + DB	Δ + DB	%Δ + DB
		(mm Hg·s^{-1})		(ml·min^{-1}·g^{-1})		(ergs·g^{-1}, x10^{-5})		(ergs·g^{-1}·s^{-1}, x10^{-5})	
Control	Pentobarbital anesthesia	0.82 ± 0.07	46.2 ± 2.3	22.4 ± 2.9	18.6 ± 3.5	0.075 ± 0.009	23.8 ± 3.6	3.29 ± 0.32	40.7 ± 2.1
Alcoholic		0.55 ± 0.10	28.8 ± 5.7 P<0.025‡	12.0 ± 3.4 P<0.05	9.8 ± 2.9	0.041 ± 0.009 P<0.025	13.2 ± 3.0 P<0.05	2.43 ± 0.29	30.9 ± 3.6 P<0.05
Control	Cervical fracture	0.56 ± 0.07‖	32.5 ± 3.7¶	4.7 ± 6.1§	2.8 ± 4.7‖	0.018 ± 0.018¶	4.4 ± 5.4¶	1.71 ± 0.56§	22.7 ± 5.4¶
Alcoholic		0.60 ± 0.10	33.9 + 6.5	9.9 ± 3.7	7.9 ± 3.1	0.033 ± 0.011	10.5 ± 3.8	2.09 ± 0.32	27.5 ± 5.4

* Each value is the mean ± S.E.M.

† Δ + DB are absolute increases in indices in response to 0.1 μM dobutamine in the perfusate. % Δ + DB are these values expressed as percent of control (pre-dobutamine) values.

‡ P values are for the effect of alcohol consumption on the various indices. Where P is not indicated, the difference between controls and alcoholics is not significant.

§, ‖, ¶ indicate cervically-fractured control values that are significantly different from pentobarbital-anesthetized control values: §, P<0.05; ‖, P<0.025; ¶, P<0.02. There are no significant differences between cervically-fractured alcoholic values and pentobarbital-anesthetized alcoholic values.

Although our explanation for the reduced cardiac dobutamine response following cervical fracture is speculative, our data indicate that possible effects of this method of euthanasia on subsequent cell, tissue, or organ function should be considered. Indeed, it would be ironic if a method of euthanasia employed to circumvent interfering anesthetic effects were itself the source of persistent confounding effects.

Our experiments also indicate the need for a systematic study of β-adrenoceptor properties and receptor-linked processes in chronic alcoholism, with attention to the method of euthanasia and state of withdrawal of the animals from alcohol. Although there have been studies of cardiac β-adrenoceptors after alcohol consumption and withdrawal [14,26], the available data are not sufficiently comprehensive to substantiate or refute our hypothesis.

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