occulsion of DA cannot be excluded. Interestingly, the phenomenon is prevented in the presence of 10^{-6}M reserpine (fig. 1). HPLC analysis of endogenous catecholamines in the vesicle pellet (electro-chemical detection) reveals values of 90.0 ± 12.8 pM/mg protein of DA, and 39.8 ± 12.8 pM/mg protein of NE (n = 6), and reflects substantial loss of endogenous DA during vesicle isolation. Exchange with these remaining endogenous substances could not lead to the DA levels observed after 12 min in the absence of ATP.

We have recently shown that the filtration assay employed in these studies captures approx. 30% of the vesicle preparation filtered[9]. Thus, levels of ^{3}H-DA accumulation in vesicles from rat striatum at 37 °C would be in the range of 900 pmol/mg of vesicle protein, a value over 20 times that of endogenous NE in the crude vesicle pellet, and more in line with relative DA and NE levels found in rat striatum[10]. In view of the similarity of characteristics of vesicular DA accumulation in whole brain vesicles[4] and the present study with striatal vesicles, and in view of the greatly elevated level of DA accumulation in striatal vesicles (278 pM/mg protein versus 41.8 pM/mg protein in whole brain vesicles), the data suggest that vesicular DA accumulation by a whole brain vesicle preparation in an impermeant medium may reflect DA uptake by dopaminergic vesicles.

1 Acknowledgments. This work was supported by grant NS 18752 (NIH) of the United States Public Health Service. Reprint requests to J.A.R.

2 Disbrow, J.K., and Ruth, J.A., Life Sci. *29* (1981) 1989.
3 Disbrow, J.K., Gershten, M.J., and Ruth, J.A., Experientia *38* (1982) 1323.
4 Gershten, M.J., Disbrow, J.K., and Ruth, J.A., Life Sci. *33* (1983) 323.
5 Tanaka, R., Asaga, H., and Takeda, M., Brain Res. *115* (1976) 273.
6 Slotkin, T.A., Salvaggio, M., Lau, D., and Kirksey, D.F., Life Sci. *22* (1978) 823.
7 Matthaei, H., Lentzen, H., and Philippu, A., Naunyn Schmiedebergs Arch. Pharmak. *293* (1976) 89.
8 Disbrow, J.K., Gershten, M.J., and Ruth, J.A., Biochem. biophys. Res. Commun. *108* (1982) 1221.
9 Disbrow, J.K., and Ruth, J.A., submitted.
10 Holman, R.B., Angwin, P., and Barchas, J.D., Neuroscience *1* (1976) 147.

0014-4754/84/070692-03$1.50 + 0.20/0

Effects of sympathetic stimulation on ventricular refractory periods in cats with acute coronary artery ligation[1]

M.S. Gaide, R.J. Myerburg, J.S. Cameron and A.L. Bassett

Departments of Pharmacology and Medicine, University of Miami School of Medicine, Miami (Florida 33101, USA), 10 May 1983

Summary. Ventricular refractory periods shorten in the ischemic area following acute coronary artery ligation. Subsequent bilateral sympathetic nerve stimulation reduces disparity in refractory periods across normal, border (peri-ischemic) and ischemic areas.

A higher incidence of cardiac arrhythmias occurs during coronary artery occlusion and sympathetic stimulation than during coronary occlusion alone[2-4]. Increased dispersion of ventricular refractory periods induced by sympathetic activation[5] has been suggested as a mechanism for the enhanced arrhythmogenesis. Recently, Burgess and Haws[6] reported the first data on the effects of sympathetic stimulation on ventricular refractoriness in the acutely ischemic canine heart and found that heightened sympathetic tone reduces inequalities in refractoriness by prolonging refractory periods in ischemic sites while shortening them in non-ischemic sites. The present results confirm their finding that sympathetic activity reduces disparity in refractory periods in the acutely ischemic heart, although the effects of sympathetic stimulation on ischemic tissue noted by us were opposite to those described by Burgess and Haws[6].

Materials and methods. Experiments were carried out in adult cats (2.0–4.7 kg) maintained under sodium pentobarbital (30 mg/kg, i.p. and supplemental doses) anesthesia and ventilated. Blood gasses and temperature were maintained in the normal range. In each cat, the sinus node was crushed and the right atrium and posterior surface of the left ventricular free wall (near the base) were paced with 2-msec pulses delivered at 1.5 times threshold voltage. Pacing cycle length was held constant for each cat and ranged from 301–350 msec for the cats studied. Myocardial ischemia identified by discrete epicardial cyanosis was induced by single-stage ligation of distal tributaries of the left anterior and circumflex coronary arteries as described previously[7,8] and allowed to evolve for 1 h. The same procedure was followed for sham-operated control cats except that non-occlusive ties were used. Epicardial bipolar electrodes were used to deliver extra-stimuli and to record surface electrograms in 3 areas on the left ventricle in each ligated heart: 1) center of the ischemia (ischemic area), 2) area bordering the ischemia (peri-ischemic or border area), and 3) normal tissue proximal to the ischemia (normal area)[7,9]. Epicardial areas studied in sham-operated control hearts corresponded to the ischemic, border and normal areas in ligated hearts and are referred to as distal, central and proximal control areas, respectively. To measure refractory periods, a train of 10 pacing stimuli were delivered to the right atrium (S_1) and left ventricle (S_2), with an S_1–S_2 interval (A–V delay) of 60–90 msec. S_2 was followed by a late diastolic extrastimulus (S_3) delivered to 1 of the 3 epicardial areas. The S_2–S_3 coupling interval then was shortened in 2 msec decrements until failure to capture occurred. The S_2–S_3 interval then was increased by 10 msec, and shortening was repeated until failure to capture occurred a second time. The refractory period was defined as the longest S_2–S_3 interval at which S_3 failed to capture the ventricle. Extrastimuli were 2-msec pulses, delivered at 2 or 15 times late diastolic threshold. Bilateral sectioning of the vagi, and decentralization of the stellate ganglia (only attachments to the right and left anterior and posterior ansae subclaviae were left intact) was carried out 1 h after coronary ligation or sham-operation. Bilateral sympathetic nerve stimulation was accomplished by delivering 4-msec pulses at 15 Hz and 2–10 mA to

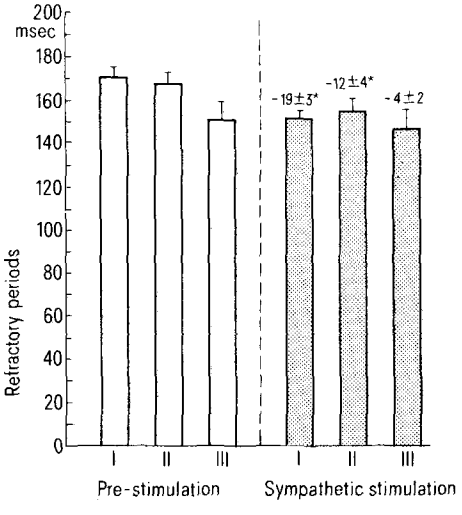
the ansae subclaviae. Analyses of variance with repeated measures[10], Duncan's Multiple Range Test[11] and F-Tests for Simple Effects[11] were used to test for significant differences in the data. Differences were considered significant at $p \leq 0.05$.

Results and discussion. Control refractory periods measured just prior to initiation of bilateral sympathetic nerve stimulation were 171 ± 5 msec ($\bar{x}$ ± SEM, proximal control area), 175 ± 6 msec (central control area) and 176 ± 4 msec (distal control area) in the 9 sham-operated hearts, and 171 ± 4 msec (normal area), 168 ± 4 msec (border area) and 151 ± 8 msec (ischemic area) in the 10 coronary ligated hearts. Refractory periods measured in the ischemic area of ligated hearts were significantly shorter ($p < 0.025$) than those measured in the corresponding area in sham-operated hearts. Refractory periods shortened uniformly and significantly ($p \leq 0.025$) from control during sympathetic stimulation in the proximal control (−9 ± 2 msec), central control (−11 ± 2 msec) and distal control (−10 ± 1 msec) areas in sham-operated hearts. In contrast, a marked heterogeneity in refractory period shortening occurred during stimulation in coronary ligated hearts. Refractory periods shortened significantly ($p \leq 0.025$) from control in the proximal normal (−19 ± 3 msec) and border (−12 ± 4 msec) areas but not in the ischemic area (−4 ± 2 msec). During sympathetic stimulation, normal area shortening was significantly ($p < 0.001$) greater, and ischemic area shortening was significantly ($p < 0.025$) less in ligated hearts compared to the shortening in the corresponding areas (proximal control and distal control, respectively) in sham-operated hearts. The net result of the heterogeneous shortening during sympathetic stimulation in coronary ligated hearts was that refractory periods became more uniform across the normal, border and ischemic areas (fig.). Refractory periods measured at a higher extrastimulus current strength (15 times threshold) were 14 ± 3 msec shorter ($p < 0.025$) than those measured at 2 times threshold, but the pattern and magnitude of refractory period shortening during sympathetic stimulation was the same.

The present data confirm the finding of Burgess and Haws[6] that sympathetic stimulation reduces inequalities in epicardial refractory periods between non-ischemic and ischemic sites in the heart with acute myocardial ischemia. Our data differ from theirs however, in that they found a sympathetic stimulation-induced prolongation of refractory periods in ischemic areas and a slight tendency for greater shortening in non-ischemic areas. In contrast, we noted minimal refractory period shortening in ischemic tissue and markedly enhanced shortening of refractory periods in non-ischemic tissue during stimulation. The difference in the effects of sympathetic stimulation may relate to the fact that the autonomic nerves apparently were intact and cardiac sympathetic nerve branches were stimulated in the Burgess and Haws[6] study, whereas the vagi were sectioned, the stellate ganglia were decentralized, and the ansae subclavia were stimulated in our study. Thus, interruption of neural reflex pathways in our study may have eliminated an inhibitory influence of the vagal nerves on norepinephrine release from sympathetic nerve terminals[12].

Our finding of enhanced refractory shortening in the normal area of ligated hearts during sympathetic stimulation may reflect an increase in β-adrenergic receptor number which was uncovered in the absence of intact reflex pathways. Catecholamine loss from normal, non-ischemic tissue begins almost immediately after coronary occlusion[13]. Also, β-adrenergic receptor number increases 1 h after coronary occlusion, at least in ischemic tissue[14]. We speculate that sufficient catecholamine was lost in the normal area of ligated hearts to stimulate an increase in β-receptor number. This, in turn, would facilitate greater responsiveness to catecholamine released from sympathetic nerves during stimulation. The inhibitory influence of vagal nerves on catecholamine release[12] may have partially 'masked' the effect of increased β-receptor number in the Burgess and Haws[6] study.

Our data and those of Burgess and Haws[6] suggest that a reappraisal of the interactions between sympathetic nerve activity and cardiac refractoriness during myocardial ischemia is warranted since sympathetic-induced disparity in ventricular refractory periods is not necessarily invariate following coronary artery occlusion.



Distribution of refractory periods measured at 2 times late diastolic threshold in the normal (I), border (II) and ischemic (III) areas prior to (left panel) and during (right panel) bilateral sympathetic nerve stimulation in coronary ligated hearts (n = 10). Refractory periods are expressed as mean ± SEM in msec. The change in refractory period brought about by sympathetic stimulation in each area is shown above the appropriate bar as mean change from control (pre-stimulation) ± SEM in msec; *, $p \leq 0.025$ (Stimulation vs pre-stimulation).

1 This work was supported by NIH Research Grants HL 19044 and HL 21735, NIH Biomedical Research Support Grant DRR No. S07-05363 and a Grant-in-Aid from the American Heart Association, Florida Affiliate, Broward County Chapter. Dr Gaide is the recipient of NIH New Investigator Research Award HL 27680.

2 Harris, A.S., Otero, H., and Bocage, A.J., J. Electrocardiol. *4* (1971) 34.
3 Kliks, B.R., Burgess, M.J., and Abildskov, J.A., Am. J. Cardiol. *36* (1975) 45.
4 El-Sherif, N., Circulation *58* (1978) 103.
5 Han, J., and Moe, G.K., Circulation Res. *14* (1964) 44.
6 Burgess, M.J., and Haws, C.W., J. Electrocardiol. *15* (1982) 1.
7 Myerburg, R.J., Gelband, H., Nilsson, K., Sung, R.J., Thurer, R.J., Morales, A.R., and Bassett, A.L., Circulation Res. *41* (1977) 73.
8 Myerburg, R.J., Bassett, A.L., Epstein, K., Gaide, M.S., Kozlovskis, P., Wong, S.S., Castellanos, A., and Gelband, H., Circulation Res. *50* (1982) 386.
9 Gaide, M.S., Myerburg, R.J., Kozlovskis, P.L., and Bassett, A.L., Am. J. Physiol. Heart Circulatory Physiol., in press.
10 Dixon, W.J., in: Biomedical Computer Programs, p. 711, Ed. BMDP. University of California Press, Los Angeles 1975.
11 Bruning, J.L., and Kintz, B.L., in: Computational Handbook of Statistics, p. 117. Scott, Foresman and Co., Atlanta 1968.
12 Muscholl, E., Am. J. Physiol. *239* (1980) H713.
13 Sakai, K., and Abiko, Y., Jap. Circul. J. *45* (1981) 1250.
14 Mukherjee, A., Wong, T.M., Buja, L.M., Lefkowitz, R.J., and Willerson, J.T., J. clin. Invest. *64* (1979) 1423.

0014-4754/84/070694-02$1.50 + 0.20/0