Avicel have been reported by Pesonen & Paronen (1986). Emcocel was reported to have a wider size distribution than Avicel but its numerical mean diameter of 11·7 μm was similar to the 12·5 μm obtained for Avicel. However, the volumetric mean diameter was reported to be much larger, 22·2 μm for Emcocel against 13·4 μm for Avicel. It is likely that the volumetrically large Emcocel particles have reduced interparticulate points of contact and thus the powder bed may show a lower degree of cohesiveness for this reason.

As the amount of granulating liquid is increased beyond 20%, the shear stress at failure decreased for both Emcocel and Avicel (Fig. 3). This behaviour of the powder bed corresponds to the well-established behaviour of the powder mass undergoing wet granulation. When the water content in the powder mass is sufficiently high to engulf much of the particle surface, the advantageous lubricating effect of the liquid is exhibited thereby promoting flow of the particulate material with significantly reduced frictional forces.

Analysis of the cohesiveness of a powder bed using the sandwich rheometer method appears to be a simple method for characterizing a powder bed and has the ability to determine both the static and dynamic behaviour of the powder bed, providing the variables are standardized.

References

Hidaka, J., Miwa, S. (1981) Kagaku Kagaku Ronbunshu 7: 184–190
Pesonen, P., Paronen, P. (1986) 5th Pharm. Technol. Conf., Harrogate, UK
Rudnic, E. M., Chilamkurti, R., Rhodes, C. T. (1980) Drug Rev. Ind. Pharm. 6: 279–289
Staniforth, J. N., Baichwal, A. R., Hart, J. P., Heng, P. W. S. (1987) 6th Pharm. Technol. Conf., Canterbury, UK
Yuasa, Y., Yamashiro, M. (1985) Kyokaishi 58: 200–203

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Presynaptic changes promoted by alloxan diabetes in the cat isolated heart

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Abstract—Adrenergic presynaptic functions were evaluated in the cat isolated perfused heart preparation. The sympathetic nerve endings were labelled with [^{3}H]noradrenaline ([^{3}H]NA) and the effect of electric neural stimulation was determined in the presence of drugs which inhibit neuronal or extraneuronal uptake, or which antagonize α-adrenoceptors. [^{3}H]NA overflow was measured in control and diabetic cats and was significantly increased by electric neural stimulation on both conditions. Perfusion with 0·1 μM phentolamine increased transmitter overflow in control hearts but failed to do so on organs obtained from alloxan-treated cats. The data provide evidence that in alloxan diabetic cats there is an abnormality of the adrenergic synapse.

Experimental diabetes has been shown to induce changes in adrenoreceptor mechanisms (Costa e Forti & Fonteles 1979; Brody & Dixon 1964). In human adipose tissue Arner & Ostman (1976) have demonstrated that treated diabetic patients presented evidence for increased α- as well as β-adrenoreceptor sensitivity. Cseuz et al (1973), using rabbit aorta, found an increase in sensitivity, though with a lack of specificity for the adrenoreceptor blockers, since α-adrenotropic effects could also be blocked by propranolol. Owen & Carrier (1980) observed supersensitivity too, and found that the vascular alterations observed in rat aortas from diabetic animals were related to the duration of diabetes and to the extracellular calcium concentration.

Szentivanyi & Pek (1973) demonstrated an increase in α-adrenergic response in conjunctival vessels of diabetic patients and Christlieb et al (1976) observed an increase in systemic blood pressure elicited by noradrenaline (NA). The same kind of observations were documented by Foy & Lucas (1976) from rat blood pressure data. On the other hand, Fonteles & Matheny (1979) observed a reduced sensitivity to NA in the iris dilator muscle preparations of diabetic animals. Williams et al (1983) observed in streptozocin diabetic rats, a decrease in density of α- and β-adrenoceptors in the myocardium.

The above observations, coupled with those of Kunos et al (1973) and Ahlquist (1977), which demonstrated that the metabolic rate could quantitatively regulate the adrenoceptors, led us to investigate α-presynaptic adrenoceptors in diabetic cats.

Methods

Cats, 1·5 to 3·0 kg were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.p.) and heparinized with 2500 u kg^{-1} through the femoral vein. The animal was intubated during surgery under artificial ventilation by means of a Harvard respiratory pump, model 680. The heart was isolated, the sympathetic nerves to the organ intact, and the cardioaccelerator nerve carefully dissected according to the technique of Hukovic &

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Muscholl (1962). The coronary vessels were perfused according to the Langendorff's method. The system was made according to Sen et al (1976). The Krebs-Henseleit perfusion fluid was of the following composition (mM): NaCl 11·8; $CaCl_2$ 2·6; H_2 $NaPO_4$ 1·0; KCl 4·7; $NaHCO_3$ 25; glucose 11·1; EDTA 4 and ascorbic acid 0·11. To avoid cholinergic interference in the system, atropine sulphate, 1·48 mM, was added to the perfusion solution.

The right sympathetic nerve was stimulated with a bipolar platinum electrode using a Grass stimulator (Model S 4K), with a square pulse of 0·5 ms and 8 V as described by Starke & Montel (1973).

After 30 min of perfusion, [^{3}H](N)(-)-NA (New England Nuclear, Boston, Mass., 21·4 Ci mol^{-1}) was administered by the aortic cannulae for 40 min at a rate of 1·25 μCi min^{-1}. The heart was then perfused for an additional 40 min before electrical stimulation. Cardiac contractility was monitored by means of a mechanical transducer coupled to a polygraph (Gilson model S 6H). The coronary flow varied between 17–21 mL min. Seven samples from the venous effluent were collected at 1 min intervals. Two samples were taken before the period of nerve stimulation, one during the electrical stimulus and four after. The tritium content of each sample (2 mL) was determined by the addition of 10 mL of Bray's solution and by reading it in a Packard spectrometer scintillation counter. Quenching was determined by an internal standard.

The diabetic condition was induced by injecting alloxan (150 mg kg^{-1} i.v.) and the animals used had a glucose level higher than 175 mg% determined by the glucose oxidase method of Washko & Rice (1961). To avoid a high rate of mortality among the cats treated with alloxan, the total dose of the drug was divided into two injections of 75 mg kg^{-1}, at intervals of 2 h. Histological examination of the pancreas of treated animals demonstrated degranulation of beta cells.

Drugs used in this study were: 0·1 μM desipramine, 2 nM normetanephrine, 0·1 mM phentolamine and 1·48 mM of atropine. Normetanephrine, atropine and alloxan were from Sigma Chemical Co. (St. Louis, Mo); desipramine and phentolamine from Ciba-Geigy.

The data were analysed by using the unpaired Student's t-test. Differences between the control and diabetic groups of animals were considered significant when $P < 0.05$.

Results

Neural stimulation induced a significant overflow of [^{3}H]NA in both control and diabetic animals. As it should be expected this promotes an increase in the contractile strength of the heart of the order of more than 200%. The basal liberation of [^{3}H]NA was significantly greater in the diabetic cats when compared with controls (Table 1) ($P < 0.001$), though phentolamine promotes a basal decrease on both control ($P < 0.01$) and diabetic preparations ($P < 0.05$).

The administration of phentolamine in the presence of desipramine caused a significant ($P < 0.01$) increase in the transmitter overflow after neural sympathetic stimulation of the heart in normal animals. Phentolamine did not promote any change on either the chronotropic or the inotropic responses of the hearts from either normal or diabetic preparations. There is a slight tendency for a decrease of these parametes in the diabetic hearts but the data is not statistically significant. However, in the diabetic group phentolamine did not cause "overflow" (Table 1). As it can be seen, after its administration there was a drop in the overflow, which varied from 2564 (100%) to 2·101, (82%), which is exactly the opposite of what happens to the normal cat heart.

Our data show that in alloxan diabetic cats the presynaptic adrenergic receptors of the isolated perfused heart are not responsive to phentolamine, which suggests an abnormality of this adrenergic synapse at neural sites.

Table 1. [^{3}H]NA flow induced by stimulation of the sympathetic nerve in the isolated hearts of normal and diabetic cats perfused with Krebs-Henseleit solution.

Experimental group	n	Basal[1]	"Overflow[2]"	Overflow % of control
(I) Normal animals				
(a) DMI+NMT	9	92±8	2216±236	100%
(b) DMI+NMT+PHEN	11	69±8	3074±360	138%
		$P<0.01$	$P<0.001$	
(II) Diabetic animals				
(a) DMI+NMT	7	347±45	2564±726	100%
(b) DMI+NMT+PHEN	7	271±35	2100±430	82%
		$P<0.05$	$P<0.05$	

[1] Effluent coronary samples were collected at 1 min intervals and are expressed as counts min g^{-1} in the basal period. The data shown represent the integrated [^{3}H]NA flow before and after neural stimulation.

[2] Overflow is the percent increase in relation to the basal flow of [^{3}H]NA induced by neural electric stimulus. DMI—Desipramine. NMT—Normetanephrine. PHEN—Phentolamine.

Discussion

Diabetes has been shown to promote neuropathy which is characterized by several neural dysfunctions that include autonomic variations of heart rate (Oikawa et al 1985). Several reports in the literature demonstrate that even in acute and subacute experimental models response to mediators and calcium are remarkably changed in this condition (Rosen & Schröa 1980; Bielefeld et al 1983).

As far as we know, there are no reports in the literature where presynaptic adrenoreceptors have been studied under this condition. Our research suggested an impairment in α_2-presynaptic receptors as shown by the changes in basal levels of NA liberated at functional sites. This observation is true in the presence and in the absence of phentolamine, which demonstrates clearly that this metabolic condition can be responsible for the maintenance of the neural transmission in this preparation. The condition known as microangiopathy affects several organs and is characterized by thickening of the basal membrane. We hypothesize that this preliminary observation could be related with basal membrane alterations such that they would provoke disruption of the feedback mechanism involved in adrenergic control of peripheral synaptic levels. The increase in basal liberation of [^{3}H]NA is compatible with the hypothesis that by a loss of the tonic inhibition of α_2-adrenoceptors on the liberation of NA, the basal transmitter release would tend to be greater.

It is now well established that noradrenergic transmitter release is regulated by presynaptic receptors (Westfall 1984).

Autonomic neuropathy is associated with long-term diabetes (Christensen 1979) and presynaptic changes must be considered as a cause. However in our experiments diabetes has a short duration. Perhaps we are dealing with an early change at the sympathetic level that ultimately would lead to post synaptic alterations such as those proposed by Carrier et al (1983), in rat aortae. Altered hyperaemic response in the coronary arterial bed of the alloxan diabetic dog has also been described by Koltai et al (1983). Bielefeld et al (1983) have also demonstrated an alteration of calcium metabolism in cardiac muscle of diabetic rats three months after streptozocin treatment.

Latifpour & McNeill (1984) observed by using binding studies, that ventricular α_1 receptor density was significantly decreased in diabetic rats while β- and muscarinic receptors

showed only a slight increase in their number. Recent evidence suggests that various biochemical changes are responsible for altered myocardial function in diabetes (McNeill & Tahiliani 1986) which includes depression of enzymes such as the Na^+, K^+-ATPase and Ca^{2+}-ATPase.

References

Ahlquist, R. P. (1977) Adrenoceptor sensitivity in disease as assessed through response to temperature alteration. Federation Proc. 36: 2572-2574

Arner, P., Ostman, J. (1976) Abnormalities in the adrenergic control and the rate of lipolysis in isolated human subcutaneous adipose tissue in Diabetes Mellitus. Diabetologia 12: 593-599

Bielefeld, D. R., Pace, C. S., Boshell, B. R. (1983) Altered sensitivity of chronic diabetic rat heart to calcium. Am. J. Physiol. 245: 560-567

Brody, M. J., Dixon, R. L. (1964) Vascular reactivity in experimental Diabetes Mellitus. Circ. Res. 14: 494-501

Carrier, G. O., Jackson, C. V., Scarborough, N. L. (1983) Influence of Diabetes Mellitus and aging on adrenergic responses of innervated and noninnervated vascular smooth muscle. Vascular neuroeffector mechanisms. 4th International Symposium. Ed. John A. Bevan, Fujiwara, M., Maxwell, R. A., Mohri, K., Shibata, S. and Toda, N. Raven Press, New York. pp 125-128

Christensen, N. H. (1979) Catecholamines and Diabetes Mellitus. Diabetologia 16: 211-224

Christlieb, A. R., Janka, H. U., Kraus, B., Gleason, R. E., Icases-Cabral, E.A., Aiello, L.M., Cabral, B.V., Solno, A. (1976) Vascular reactivity to angiotensin II and to norepinephrine in diabetic subjects. Diabetes 25: 268-274

Costa e Forti, A., Fonteles, M. C. (1979) An alpha-adrenotropic study of the normal and diabetic rabbit kidney. Arch. Int. Physiol. Biochem. 87: 887-897

Cseuz, R., Wengner, T. L., Kunos, G., Szentivanyi, M. (1973) Change of adrenergic reaction pattern in experimental Diabetes Mellitus. Endocrinology 93: 752-755

Fonteles, M. C., Matheny, S. L. (1979) Alpha-adrenoceptor alterations by alloxan diabetes in iris dilator muscle of the rabbit. Experientia 35: 6333

Foy, J. M., Lucas, P. D. (1976) Effect of experimental diabetes, food deprivation and genetic obesity on the sensitivity of the pithead rats to autonomic agents. Br. J. Pharmacol. 57: 229-234

Hukovic, S., Muscholl, E. (1962) Die Noradrenalin-abgabe aus dem isolierten Kaninchenherzen bei synpathischer Nerwenreizung und ihre pharmakologis che Beeinflussung. Naunyn Schmiedeberg's Arch. Pharmacol. 244: 81-96

Koltai, M. Zs., Wagner, M., Pogatsa, G. (1983) Altered hyperremic response of the coronary arterial bed in alloxan diabetes. Experientia 39: 738-740: 198

Kunos, G., Yong, M. S., Nickerson, M. (1973) Transformation of adrenergic receptors in the myocardium. Nature New Biology 241: 119-120

Latifpour, J., McNeill, J. H. (1984) Cardiac autonomic receptors effect of long term experimental diabetes. J. Pharmacol. Exp. Ther. 230: 242-249

McNeill, J. H., Tahiliani, A. G. (1986) Diabetes-induced cardiac changes. Trends Pharmacol. Res. 7: 364-367

Oikawa, N., Umetsu, M., Toyota, T., Goto, Y. (1985) Quantitative evaluation of diabetic autonomic neuropathy by using heart rate variations determinations of the normal range for the diagnosis of autonomic neuropathy. J. Exp. Med. 145: 233-241

Owen, M. P., Carrier, G. O. (1980) Calcium dependence of norepinephrine induced vascular contraction in experimental diabetes. J. Pharmacol. Exp. Ther. 212: 253-258

Rosen, P., Schröa, K. (1980) Increased prostacyclin release from perfused hearts of acutely diabetic rats. Diabetologia 18: 391-394

Sen, A. K., Sunarara, F. A., Talesnia, J. (1976) Prostaglandin E_2 and cyclic AMP in the coronary vasodilation due to cardiac hyperactivity. Can. J. Physiol. Pharmacol. 54: 128-139

Starke, K., Montel, R. (1973) Sympathomimetic inhibitors of noradrenaline release mediated by prostaglandins. 278: 111-116

Szentivanyi, M., Pek, L. (1973) Characteristic changes of vascular adrenergic reactions in Diabetes Mellitus. Nature New Biology 243: 276-277

Westfall, T. C. (1984) Evidence that noradrenergic trnsmitter release is regulated by presynaptic receptors. Fed. Proc. 43: 1352-1357

Washko, M. E., Rice, E. W. (1961) Determinations of glucose by an improved enzymatic procedure. Clin. Chem. 7: 542-545

Williams, R. S., Schaible, T. F., Scheuer, J., Kennedy, R. (1983) Effects of experimental diabetes on adrenergic and cholinergic receptors. Diabetes. 32: 881-886