Neural Regulation of Glucose-Stimulated Insulin Secretion in Younger and Older Fischer 344 Rats (43238)

DONALD L. CURRY* AND ROGER B. MCDONALD[†,1]
Departments of Physiological Sciences and Nutrition,[†] University of California, Davis, California 95616*

Abstract. **Neural regulation of insulin secretion of *in situ* innervated perfused pancreases was evaluated in younger (5 months) and older (26 months) Fischer 344 rats. In one protocol, the central nervous system (CNS) was intact throughout the entire 120-min perfusion period. In the other protocol, the CNS was intact only through the first 20 min of the 120-min perfusion, whereupon the CNS was ablated via anoxia. In both protocols, a modified Krebs-Ringer buffer containing glucose at 200 mg/dl was perfused through the pancreas at a rate of 4.8 ml/min by using a constant flow perfusion pump. Insulin secretion (ng · min^{-1}) of younger and older CNS-intact rats did not differ significantly. After the ablation of the neural regulation of the pancreas, glucose-stimulated insulin secretion of younger rats was significantly lower, relative to the average insulin secretion before ablation (i.e., min 1–20) of CNS-intact animals. This would suggest that the nature of neural control of insulin secretion in younger rats is potentiation. In contrast, insulin secretion of older CNS-ablated animals was similar, or generally increased, when the data were expressed either on an absolute or a relative basis to preablation values, respectively. Thus, these data suggest that the neural regulation of glucose-stimulated insulin secretion in younger versus older rats is significantly different.**

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Impaired endocrine pancreatic function has been associated with the aging process (1–6), however, the relationship between aging and suboptimal pancreatic function is not clear. For example, some investigators have reported a significant decline in overall glucose-stimulated insulin release by pancreases of aging rats (3–5). Others have reported that pancreases of older animals exhibit a decline in insulin only when secretion is expressed relative to the total amount of islet tissue present (6, 7), even though the total amount of insulin released by aged pancreases may be equivalent to, or even greater than, that secreted by pancreases from younger animals. Furthermore, in C57BL/6J mice (8) and Sprague-Dawley rats (9), glucose tolerance was not attenuated in older versus younger animals. Thus, some questions remain concerning the effect of aging on insulin secretion.

Studies describing mechanisms accounting for the apparent age-related decline in insulin secretion have focused on cellular dysfunction. These studies have concluded that age-related impairment of insulin secretion is due to disruption of protein transcription (10), paracrine regulatory mechanisms (11, 12), or disruption of vesicle migration to the plasma membrane of the secretory cell (5). However, because insulin secretion involves many levels of control (i.e., humoral, hormonal, hormonal-paracrine, intercellular junctions, and neural), it is unlikely that attenuated insulin secretion in aging mammals can be solely attributed to cellular events. It is possible that more than one age-related level of regulation could explain the age-related decline of endocrine pancreatic function.

One possible age-related alteration in the regulation of normal insulin secretion may be via the central nervous system (CNS). The CNS has the potential to exert substantial control over insulin secretion, either indirectly by hormonal control, such as adrenal medullary discharge, or directly via innervation to the endocrine pancreas (13). For example, sympathetic nervous system stimulation (14–16) or catecholamine administration (14, 16) inhibits ongoing insulin secretion. Conversely, parasympathetic nervous system stimula-

[1] To whom requests for reprints should be addressed at Department of Nutrition, University of California, Davis, CA 95616.

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tion via the vagus nerve (17), as well as parasympathomimetic agents (18), potentiate glucose-stimulated insulin secretion. We have recently shown that the CNS exerts a direct tonic neural inhibition of insulin secretion (19), which is perhaps associated with a reflex loop that has an afferently mediated vagus nerve component (20).

The purpose of this investigation was to determine whether altered CNS control of insulin secretion may play a role in age-related endocrine pancreas dysfunction. To this end, we have measured glucose-stimulated insulin secretion of younger and older rats by using an *in situ* brain-pancreas perfusion system.

Materials and Methods

Male Fischer 344 (F344) rats ages 5 and 26 months were used in this investigation. They were obtained from the National Institute on Aging's rodent colony (Harlan Sprague-Dawley Laboratories, Indianapolis, IN). On arrival, the animals were placed in laminar flow barrier units that provided filtered air. Maintenance of the barrier units and health screening procedures for the colony have been described (21). All rats were individually housed in wire-bottom hanging cages (20 × 25 × 18 cm), maintained on a 12:12-hr light:dark cycle (lights on at 0600 and off at 1800 hr) at a room temperature of 25–26°C, fed NIH-31 chow (Teklad Research Diets, Indianapolis, IN) *ad libitum*, and given free access to acidified distilled water (pH 3.5).

The use of two age groups (young, 5 months and adult, 26 months) rather than three (young, 5 months; intermediate, 12 months; and old, 26 months) is based, in part, on previous observations that insulin secretion of *in vitro* perfused pancreases are not generally different among younger and older rats (22). That is, total insulin released during the 2-hr perfusion did not differ significantly among the age groups (6 months, 30.6 ± 3.9 μg; 12 months, 30.6 ± 2.3 μg; 26 months, 32.1 ± 4.1 μg). Therefore, under the conditions of this initial investigation evaluating the possible alterations in neural control of the pancreas during aging, an intermediate age group was not included.

Surgical Procedures. The *in situ* brain-pancreas experimental model was used in these studies, and the complete description of the surgical procedures, validity, and reliability of this perfusion technique has been described elsewhere (19, 20, 23). In summary, after anesthesia by intraperitoneal administration of chloropent (32 mg/kg), the abdominal cavity was exposed and the adrenal glands were ligated. The latter step minimizes the possibility of surgical stress-induced release of catecholamines and their subsequent inhibition of insulin secretion. The functioning cephalad portion of the animal was vascularly isolated from the caudad end of the animal at the level of the diaphragm. The respiratory center continued to drive normal respiration and the cardiac muscle maintained normal blood flow to the cephalad section, thus providing oxygenated blood to maintain brain activity (19). The pancreas and adjoining organs (stomach, spleen, and proximal duodenum) were retained *in situ* for perfusion. Extreme care was taken not to disturb the celiac ganglion and autonomic or mixed pancreatic nerves. The pancreas and associated tissues were perfused via an arterial cannula placed in the celiac artery and drained by means of a venous cannula placed in the portal vein at its entry into the liver. All other caudal tissues not perfused were allowed to expire by anoxia. The resultant experimental model is an *in situ* innervated pancreas that is cross-perfused with exogenous buffer and coupled with an intact cephalad portion of the animal, which receives its endogenous blood supply via normal cardiopulmonary circulation.

A modified Krebs-Ringer buffer (24) containing glucose at 200 mg/dl was perfused at a rate of 4.8 ml/min using a constant flow perfusion pump (Cole-Parmer Masterflex, Chicago, IL). Pump pressure was continually monitored to detect any vascular resistance changes within the perfused tissues. Tissue temperature was maintained at 37°C by use of an external heat source and warmed (37°C) perfusate. The preparations were perfused for 2 hr and total venous effluent was collected over the following time periods: minutes 1, 2, 3 . . . 10, 13, 16, 19, 20, 21 . . . 30, 35, 40, 50, 60 . . . 120. Flow rate was recorded and perfusates were stored at −20°C for subsequent insulin determinations (25).

Experimental Protocols. Two experimental protocols were used in this investigation. In one protocol, the CNS was intact (CNSINT) throughout the entire 2-hr perfusion period. This protocol served to establish the amount of insulin secreted in response to a high physiologic glucose concentration (200 mg/dl) by pancreases of both younger and older rats while under CNS control. In the other protocol, the CNS was intact only through the first 20 min, whereupon the CNS was ablated via anoxia (CNSABT). During the remainder of the 2-hr perfusion period, the pancreases were no longer subject to CNS control.

Because of the normally high biologic variation of insulin secretion associated with perfusion methodology, we have expressed our data in terms of absolute amount secreted (ng · min^{-1}; Fig. 1) during the 120-min perfusion, as well as a percentage of the total insulin secreted relative to the first 20-min perfusion (Table I). The expression of insulin secretion after ablation relative to this 20-min period enabled us to minimize the biologic variation by having each preparation serve as its own internal control. When the data were analyzed using min 13–20 or the corresponding time points of the CNSINT as the control period for ablation, the results were similar (data not presented).

Statistical Comparisons. One-way analysis of var-

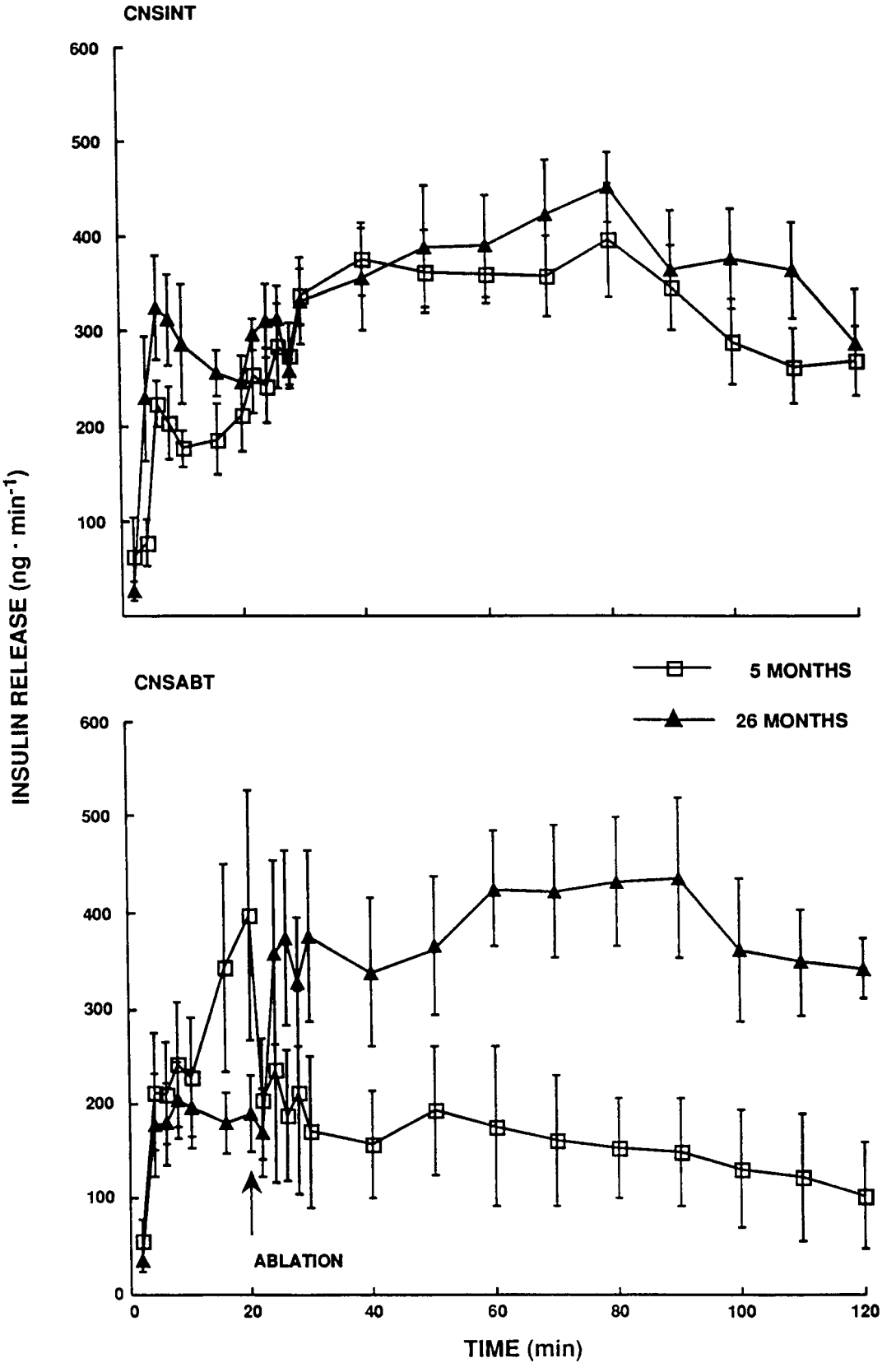

Figure 1. Glucose-stimulated (200 mg · dl^{-1}) insulin secretion (mean ± SE) of CNSINT and CNSABT younger and older F344 rats.

Table I. Percentage of Change in Glucose-Stimulated (200 mg·dl^{-1}) Insulin Secretion of CNS Intact and Ablated Perfused Pancreas Relative to Min 1–20[a]

Time (min)	CNS intact		CNS ablated	
	5 Months ($n = 5$)	26 Months ($n = 5$)	5 Months ($n = 6$)	26 Months ($n = 6$)
20–30	−20.0 ± 8.5[a,b]	−32.6 ± 5.5[a,b]	−49.6 ± 8.8[b]	−11.4 ± 11.2[a]
30–40	−8.4 ± 12.5[a]	−11.9 ± 12.9[a,b]	−60.5 ± 11.7[b]	18.4 ± 20.2[a]
40–50	19.3 ± 13.7[a]	−10.3 ± 17.1[a]	−54.5 ± 10.9[b]	27.0 ± 13.2[a]
50–60	12.6 ± 11.0[a,c]	−10.3 ± 18.2[a]	−57.8 ± 10.1[b]	49.2 ± 18.5[c]
60–70	13.6 ± 12.5[a]	−4.0 ± 9.6[a,b]	−59.9 ± 9.6[b]	43.3 ± 35.2[a]
70–80	19.5 ± 24.1[a,c]	0.8 ± 7.8[a]	−60.2 ± 10.2[b]	58.3 ± 23.7[c]
80–90	16.7 ± 12.5[a,c]	−7.9 ± 9.6[a]	−65.6 ± 7.4[b]	57.6 ± 21.7[c]
90–100	7.8 ± 14.8[a,c]	−19.9 ± 12.6[a]	−66.9 ± 7.9[b]	16.3 ± 11.5[c]
100–110	−12.8 ± 9.5[a]	−13.2 ± 14.5[a]	−71.2 ± 8.5[b]	25.9 ± 28.3[a]
110–120	−17.4 ± 13.0[a]	−19.8 ± 8.5[a]	−74.1 ± 6.1[b]	28.5 ± 28.0[a]

[a] Values are mean ± SE. Values for 5- and 26-month CNS intact rats at 30–40 through 110–120 min are not significantly different from zero.
[b] Within a row, values not sharing a common letter are significantly different ($P < 0.05$).

iance was used to evaluate possible differences between CNSINT and CNSABT, younger and older rats. When a significant effect was found, Scheffe's post hoc test was used to determine differences among the groups. Differences were considered significant at $P < 0.05$.

Results

Body and Pancreas Weight. Body weight was significantly greater in older rats, but did not differ among CNSINT and CNSABT rats of similar age (younger: CNSINT, 343 ± 8 g; CNSABT, 364 ± 12 g; older: CNSINT, 428 ± 9 g; CNSABT, 416 ± 9 g). Pancreas weight did not differ significantly among the groups (younger: CNSINT, 1.15 ± 0.07 g; CNSABT, 1.20 ± 0.15 g; older: CNSINT, 1.00 ± 0.10 g; CNSABT, 1.13 ± 0.12 g).

Insulin Secretion. In general, glucose-stimulated insulin secretion, ng · min^{-1} of younger and older CNSINT rats did not differ significantly during the 2-hr perfusion period (Fig. 1). In contrast, younger CNSABT versus younger CNSINT and older CNSABT, rats had significantly less insulin secretion after the ablation of neural innervation of the pancreas (Fig. 1). When the insulin secretion data were expressed as ng · min^{-1}, older CNSABT and CNSINT rats did not differ significantly.

Table I shows that when insulin secretion data are expressed relative to the average amount released during min 1–20, younger CNSABT rats secreted significantly less insulin than did the younger CNSINT and older CNSABT rats. Older and younger CNSINT rats did not differ significantly. The percentage of change in insulin secretion was consistently greater in older CNSABT versus CNSINT rats, and significant differences were observed at 50–60, 70–80, 80–90, and 90–100 min.

Vascular Pressure. The mean perfusion pump pressure (mm Hg) required to maintain a flow rate of 4.8 ml/min in younger and older CNSINT rats did not differ throughout the 120-min perfusion (Fig. 2). There was a significant increase in pressure after ablation in both CNSABT groups. This increase reached a peak at 3 min of ablation (younger, 161 ± 26 mmHg; older, 161 ± 25 mm Hg) and then returned to the preablation values 8 min after ablation (younger, 58 ± 10 mmHg; older 47 ± 7 mm Hg). Pressure did not differ significantly for the remainder of the perfusion in the CNSABT groups.

Discussion

Several investigators have concluded that attenuated insulin secretion, expressed on a islet mass basis, is a normal consequence of aging (1, 5, 7, 10, 26–28). The glucose-stimulated insulin secretion data obtained from *in situ* brain-pancreas intact perfusions (Fig. 1 and Table I) indicates that the presence of neural regulation modifies this response. That is, intact regulatory systems, such as the CNS, may significantly alter total insulin secretion so as to overcome significant cellular dysfunction. Our suggestion that the level of physiologic regulation present in the secretory system is an important consideration in the interpretation of data describing the effect of aging on insulin secretion is consistent with the reports of Chaudhuri *et al.* (11). These authors reported that glucose-stimulated insulin secretion of islets isolated from 2-month-old F344 rats was significantly greated compared with 24-month-old animals. However, when somatostatin, a regulatory inhibitor of insulin secretion, was added to the incubation medium, insulin secretion did not differ significantly between the age groups. Magal *et al.* (12) also reported a similar regulatory influence of somatostatin on β-cell function in younger and older F344 rats. Thus, the data presented here would suggest that attenuated insulin secretion observed in islets isolated from aging animals is influenced by the lack of CNS control.

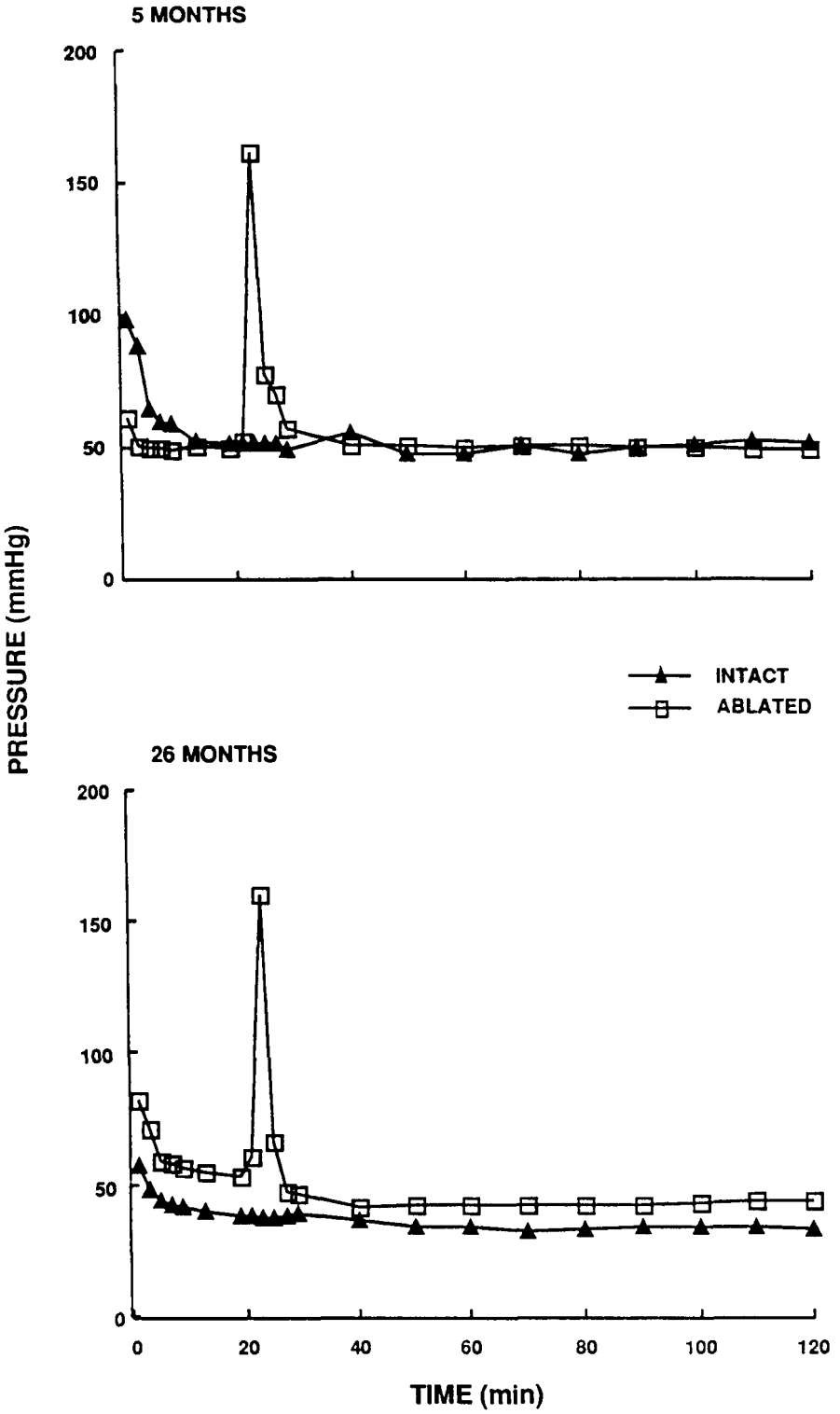

Figure 2. Mean arterial pressure of younger and older CNS intact and ablated rats generated by the constant flow pump to maintain constant flow during perfusions. The CNS was ablated (via anoxia) at min 20 of perfusions.

The possibility that regulatory factors governing insulin secretion may change with age is reflected in the insulin secretion data obtained after ablation of the CNS (Fig. 1). Ablation of the CNS in younger rats caused a significant decrease in the absolute (ng · min^{-1}) and relative (percentage of change) insulin secretion, compared with younger CNSINT animals (Fig. 1 and Table I). In contrast, insulin secretion of older CNSABT versus CNSINT rats, expressed in absolute terms, was similar (Fig. 1), but when expressed as

percentage of change, a slight increase was observed (Table I). Although the reasons for these different responses are not clear, we propose two interpretations.

First, it is possible that the neural regulation of insulin secretion of younger animals is primarily controled by the parasympathetic nervous system, compared with sympathetic regulation in the older animals. The observed decrease in insulin secretion by younger CNSABT rats would indicate that the neural regulation is stimulatory and, thus, controled through the parasympathetic (vagal) pathway (13, 15, 20). On the other hand, the increase in insulin secretion observed in the older CNSABT rats (percentage of change, Table I) would suggest that the neural regulation is inhibitory, a state characterized by sympathetic control (13, 19). Thus, 5-month-old F344 rats enhance the intrinsic insulin secretory rate of their pancreases via direct autonomic nervous system activation, whereas 26-month-old rats fail to exhibit this capability of direct tonic CNS potentiation of secretion. These results are surprising, because previous investigations have suggested that aging in humans and rats is characterized by increasing skeletal muscle insulin resistance and hyperglycemia (7, 29–32). That is, it could be argued that skeletal muscle insulin resistance would lead to an elevation in, rather than an inhibition of, pancreatic insulin secretion. This argument is consistent with the data of Lee *et al.* (33), who report that hyperinsulinemia of the insulin-resistant Zucker fatty rat is directly related to the enhanced parasympathetic tone of the pancreas. On the other hand, enhanced inhibitory control of the pancreas in older rats is consistent with the generally accepted concept that sympathetic activity is greater in aging versus younger mammals (34). Furthermore, Young *et al.* (35) have shown that serum norepinephrine, the neurotransmitter of the sympathetic system, is greater in older humans during an oral glucose tolerance test when compared with younger subjects.

Second, it is also possible that tonic neural regulation of glucose-stimulated insulin secretion in older rats no longer exists. This interpretation is based on the observation that glucose-stimulated insulin secretion expressed as ng · min^{-1} (Fig. 1) was similar among older CNSINT and CNSABT rats. That is, ablation of the CNS did not significantly affect absolute values of glucose-stimulated insulin secretion of older rats. However, sympathetic neural innervation to the pancreas of the older rat appears to be intact and functioning. This fact is supported by the observation that vascular pressure increased after ablation in both younger and older CNSABT rats (Fig. 2). The only explanation for this observation is that brain asphyxia neurally induces sympathetic-mediated vasoconstriction, via norepinephrine, of the pancreatic blood vessels because there was no vascular connection between the caudad and cephalad portions of each preparation. (Pilot studies in younger rats ($n = 3$), which were designed to verify the neural innervation of this preparation, have shown that increased pump pressure after brain asphyxia is highly correlated with the appearance of norepinephrine in the venous effluent.) As expected, due to the death of the central portion of the preparation, the vasoconstriction was transient and soon ceased. In addition, every older CNSABT rat showed an increase in absolute insulin secretion after ablation. This observation is demonstrated in Table I, in which the mean percentage of change in insulin secretion of older CNSABT rats is consistently, but not always significantly, greater than that of older CNSINT animals.

The data reported here, and supported by previous investigations (8, 9), suggest that glucose-stimulated insulin secretion of whole perfused pancreases is not altered in older rats. Although it is possible that insulin secretion of β cells declines as a function of aging, we suggest that appropriate glucose-stimulated insulin release is maintained in the whole pancreas by alterations of some, possibly neural, regulatory factor. Regardless of our speculative interpretation of these data, it is clear that neural regulation of the endocrine pancreas is different between younger and older rats and warrants further investigation.

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