injection of 6-OHDA (50 μg), respectively, produced a 92% and a 80% reduction of striatal DA contents. Thus, the present evidence is in favor of the involvement of the nigrostriatal DA pathway in thermoregulation. Specifically, severe loss of pars compacta somata or substantial depletion of striatal DA contents lead to a thermoregulatory deficit in the rat in the cold (8 °C T_a). In addition, it has been shown that the depletion of extra-central noradrenaline levels with systemic administration of either 6-OHDA or alpha-methyl-p-tyrosine[11,16,17], surgical sympathectomy[18] and immunosympathectomy plus adrenal demedullation[19] caused an impairment in adaptability to changes in cold environmental temperatures. It is not known whether the nigrostriatal DA depletions result in an inhibition in both heat production and heat loss mechanisms in the cold via a reduction of the extra-central noradrenaline level or a reduction in preganglionic sympathetic outflow in the rat. In this situation, the decreased rate of cutaneous heat losses could not effectively counteract the impaired M, which led to hypothermia.

Finally, it must be acknowledged that electrolytic lesions destroy more tissue than only the pars compacta DA neurones. In addition, the high amount of 6-OHDA (50 μg) must have destroyed non-dopaminergic neurones in the neighborhood. However, the present results showed that unacceptable, electrolytic lesions to the SN produced no deficiency in thermoregulation accompanied by a slight reduction (33%) of striatal DA contents. This indicates that thermoregulatory deficit is specifically correlated with nigral DA neurones. Our recent findings also showed that the apomorphine-induced hypothermia was not enhanced by pretreatment with 6-OHDA[20]. Therefore, the supersensitivity of the residual dopamine can be ruled out in the present experiments as mediating the observed deficiency in thermoregulation.

1 This work was supported by grants from the Pjing-Ling Neurological Foundation (Veterans General Hospital, Taipei, Taiwan, China) and the National Science Council (Republic of China). The authors are grateful to Mr C.C. Wei for his generous support.

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Control of muscle insulin receptors by the motor nerve*

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Summary. I^{125} insulin binding and the uptake of oxygen and 2-deoxyglucose are increased in skeletal muscle after denervation, suggesting an increase in insulin receptors (IR). Sustained increases in the number of affinity of IR molecules may account for some properties of denervated muscle fibres.

Muscle denervation is followed by a well-known sequence of metabolic and electrophysiological changes, including an increase of glucose and amino acid uptake[1,2], more rapid protein turn-over[3], increased calcium binding by sarcolemma and sarcoplasmic reticulum[4,5], greater contractile responses to agents causing an increase in intrafibre calcium ($[Ca^{++}]$)[6-8], as well as partial depolarization[6,9], a rise in transmembrane resistance[6,9,10], reduction of potassium and chloride permeabilities (pK^+, pCl^-)[11-13], and an increase in pNa^+ [14]. There is also a remarkable spread of acetylcholine receptors (AchR) over the muscle surface[8,15-17]. Though it may initially gain weight[18], the muscle then atrophies. With the exception of depolarization and atrophy very similar changes can be produced by exposing the muscle to insulin[19-27]. We have therefore examined the effects of denervation on the insulin receptor (IR) of skeletal muscle and find that interruption of a muscle's nerve supply is followed by an increase in either the hormone binding affinity or the number of IR sites on the membrane. To estimate the number and activity of insulin-receptor complexes on muscle we have measured insulin binding (I^{125} insulin), labelled hexose uptake (^{3}H-2 deoxyglucose), oxygen consumption ($\dot{V}o_2$) and resting membrane potential (RMP) in experiments on normal or denervated soleus (SOL) or extensor digitorum longus (EDL) muscles of young, adult (75-125 g), male Wistar rats. For the insulin binding studies only SOL was used, the preparation being essentially the same as that of Le Marchand-Brustel et al.[28]. The muscles were washed for 1 h after removal and incubated 2.5-7 h in the nutrient solution of Bretag[29] with 2% BSA (fraction V, Sigma, St. Louis, MO, USA) and I^{125} insulin (New England Nuclear, Boston, MA, USA, sp. act. ~100 μCi/μg) in a final concentration of 2.5 ng/ml, or 0.4×10^{-9} M. To estimate non-specific binding, a normal or denervated muscle of similar weight from another animal was simultaneously incubated in the same solution, to which was

Table 1. Effects of denervation on insulin I^{125} binding and hexose uptake, rat soleus

Type	n	Weight	Nonspecific binding*	% of total binding	Specific binding*	% Change Denervated/ normal	2-DG-uptake**	n	Change (%)
Normal	21	59.8 ± 3.2	0.08 ± 0.01	31	0.12 ± 0.08		1.29 ± 0.13	12	
Denervated (6-7 days)	16	41.4 ± 4.8	0.09 ± 0.02	17	0.44 ± 0.10	+367	1.65 ± 0.18	12	+28

* Results as mole $\cdot 10^{-15} \cdot$ mg muscle$^{-1} \cdot h^{-1} \pm$ SD. ** Results as mole $\cdot 10^{-9} \cdot$ mg muscle$^{-1} \cdot h^{-1} \pm$ SD.

Table 2. Effects of denervation on insulin metabolic and electrophysiological responses

Muscle type	n	$\dot{V}o_2$ ($\mu l \cdot g^{-1} \cdot h^{-1}$) Control	Insulin	Membrane potential (mV) Control	n	Insulin	n
Normal EDL	15	221.8 ± 22.5	246.4 ± 18.3	74.1 ± 1.2	442	79.4 ± 3.4	211
Normal SOL	6	180.3 ± 19.8	189.7 ± 30.8	73.5 ± 4.2	125	74.2 ± 4.0	50
Denervated EDL	12	364.2 ± 25.8	302.8 ± 25.1	66.1 ± 2.8	369	67.1 ± 4.3	185
Denervated SOL	4	320.4 ± 19.8	267.5 ± 24.3	62.1 ± 6.3	100	58.6 ± 6.2	50

Insulin concentration 50 mU/ml. Values are means ± SD.

added 'cold' insulin (monocomponent porcine sodium insulin No.IDG 04-94-193, Lilly, Indianapolis, IN, USA), 10^{-6} M[30]. Hexose uptake was measured with ^3H-labelled 2 DG in 12 normal and denervated muscles. The sp.act. of the 2 DG was 40 Ci/mmole, and its concentration was adjusted to 25 µCi/ml. Incubation was 1 h at 22-24 °C. After multiple washings, the muscles were dissolved in 1.2 N NaOH, the I^{125} then being counted on a Searle automatic gamma counter (Model 1185), tritium with a Searle liquid scintillation system (Delta 300). Membrane potentials (RMP) were recorded with conventional KCl-filled glass microelectrodes, and oxygen uptake ($\dot{V}o_2$) was measured with a polarographic technic described in detail elsewhere[23]. The insulin-binding and 2-DG-uptake studies are presented in table 1. The data are from 4-h incubations, by which time specific binding appeared to have reached a steady state. It can be seen that denervation is followed by an increase in specific insulin binding without change in non-specific complexing. There is also a small rise in 2-DG-uptake. Table 2 shows the results of the electrophysiological and metabolic experiments. There is a significant elevation of RMP by insulin in normal muscles ($p < 0.02$), but this hyperpolarizing shift was not seen in the depolarized, denervated fibres. While insulin caused a mild elevation of $\dot{V}o_2$ in normal EDL and SOL muscles ($0.1 > p > 0.05$), the result in denervated preparations was to lower the high resting oxygen uptake. The data are taken to mean that denervation of muscle causes an increase in either the binding affinity or the availability of insulin ligands on the membrane. The effects on RMP and $\dot{V}o_2$ suggest that the binding sites are altered by loss of some neutrotrophic effect or that chronic exposure of the muscle in vivo to an excess of IR complexes changes the properties of the membrane. Over time, some of the effects of the hormone on the denervated muscles may have reached a maximum in vivo and are no longer affected by additional insulin exposure in vitro. This is certainly true for $\dot{V}o_2$. It is of interest that, as with synaptic receptor, there is more IR on the developing than on the mature muscle[31]. The motor nerve thus appears to influence properties or availability of membrane components regulating the muscle's energy supply in much the same way as those for synaptic transmission. It will be of interest to learn what aspect of neuromuscular function regulates the hormonal ligands.

* Supported by the Medical Research Service of the Veterans Administration.

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