The loss of hormone activity depends (though not to any proportionate extent) on the incubation time and the iodoacetate concentrate, a clearly inhibitive action becoming evident after 90 min and a concentration of $5 \cdot 10^{-5} M$ of iodoacetate.

Influence of the hormone concentrate with regard to its antigenic power stability: A study has been made of the effect of the insulin concentrate in relation to the stability of its antigenic power by exposing different samples of bovine insulin to various concentrates of iodoacetate. As will be seen from Table III, the increase in insulin concentrate to 300 μU/ml over an incubation time of 120 min fails to protect the antigenic stability of the hormone when placed in the presence of iodoacetate.

Table I. *Effect of iodoacetate on beef insulin stability*

Reagent present during incubation	Residual activity (μU/ml) at 24 h of incubation
None	100
$1 \cdot 10^{-4} M$ iodoacetate	64
$5 \cdot 10^{-5} M$ iodoacetate	85
$1 \cdot 10^{-5} M$ iodoacetate	98
$5 \cdot 10^{-6} M$ iodoacetate	97

Tubes contained 100 μU/ml of crystalline beef insulin and the indicated additions in phosphate buffer 0.040 M, pH 7.4. Incubation was carried out at 0–4 °C.

Table II. Effect of iodoacetate on beef insulin stability

Reagent present during incubation	Residual activity (μU/ml) Time (min)			
	0	30	90	120
None	200	190	175	160
$1 \cdot 10^{-4} M$ iodoacetate	–	139	70	65
$5 \cdot 10^{-5} M$ iodoacetate	–	154	92	82
$1 \cdot 10^{-5} M$ iodoacetate	–	159	106	90
$5 \cdot 10^{-6} M$ iodoacetate	–	180	170	150

Tubes contained 200 μU/ml of crystalline beef insulin and the indicated additions in phosphate buffer 0.040 M, pH 7.4. Incubation was carried out at 37 °C.

Inhibition of the antigenic power of the insulin by iodoacetate makes it clear that this reactive does not act exclusively as a blocker of the –SH groups, but also affects other structural groups necessary to the hormone's antigenic stability. MICHAELIS[8] reported in 1934 on the action of the iodoacetate on other non-sulphydric parameters of the proteins and showed the quick, easy action of this halogenated acid with the amino groups of the amino acids.

Nevertheless, HALIKIS and ARQUILLA[9], studying immunological aspects of insulin by using isothiocyanate fluorescein, came to the conclusion that the substitution of the amino groups inhibits the antigenic power of the hormone specifically.

Table III. Influence of iodoacetate on stability at several hormone concentrations

Concentration of hormone (μU/ml)	Residual activity (μU/ml) at 120 min of incubation				
	No iodoacetate	With iodoacetate $1 \cdot 10^{-4} M$	$5 \cdot 10^{-5} M$	$1 \cdot 10^{-5} M$	$5 \cdot 10^{-6} M$
150	112	68	80	84	88
200	160	65	82	90	150
300	220	100	148	180	230

Tubes contained the hormone concentration and the indicated additions in phosphate buffer 0.040 M, pH 7.4. Incubation was carried out at 37 °C.

Résumé. Nous avons observé que le iodoacétate en concentrations de $1 \cdot 10^{-4}$, $5 \cdot 10^{-5}$ et $1 \cdot 10^{-5} M$ et préalablement incubé à 37 °C, a une influence de l'ordre de 61, 50 et 44% respectivement sur la totalité du pouvoir antigénique de l'insuline de bœuf. L'effet inhibitif est proportionnel à la durée d'incubation et l'on ne constate pas de différences lorsque les concentrations de l'hormone se trouvent entre 100 et 300 μU/ml.

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Different Activation of the Two Types of the Pyramidal Tract Neurones Through the Cerebello-Thalamocortical Pathway

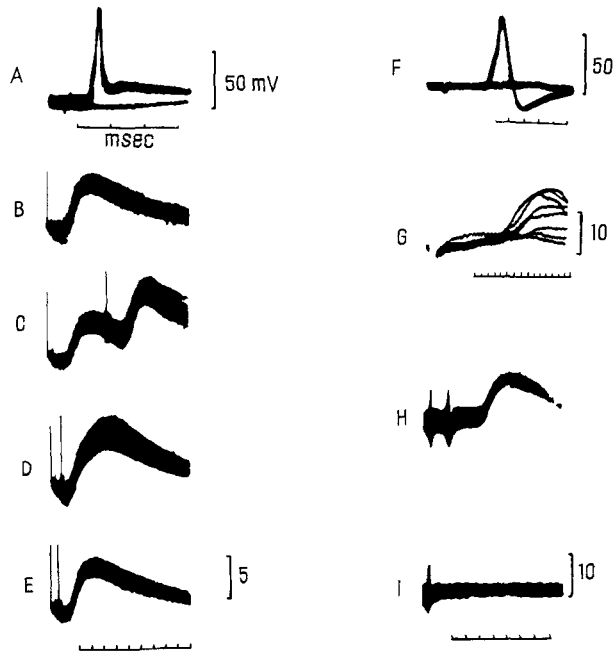
The motor area of the cat's cerebral cortex receives histologically a projection from the nucleus ventralis lateralis (VL) of the thalamus[1], which is innervated by the intracerebellar nuclei[2]. Activation of the pyramidal tract neurone (PTN) through this cerebello-thalamocortical pathway has been demonstrated by several authors[3–5]. Intracellular recording has further revealed that by stimulating VL directly EPSP's were induced from PTN's with short latency[6,7]. However, PTN's can be classified into two groups by the axonal conduction velocities in their antidromic activation: the faster group and the slower[8,9]. So we investigated the VL-evoked EPSP's with special reference to the axonal conduction velocities of each PTN, and a clear difference was found between the fast and the slow PTN's.

Cats were anaesthetized with pentobarbitone sodium (30 mg/kg). Bipolar concentric electrodes for stimulation were inserted to VL and to the brachium conjunctivum (BC) stereotaxically. After the removal of the oesophagus and the basilar bone, stimulating electrodes of enamel-

coated wires were placed on the left pyramid at about 5 mm caudal from the inferior edge of the pons. They were fixed to adjacent bone with dental cement. Glass microelectrodes were filled with solution of 3 *M* KCl or 2 *M* K-citrate (10–20 *M* Ω) and were fed to a transistorized high-impedance input circuit[10]. PTN's were identified by their antidromic excitation through pyramidal tract[11].

In a fast PTN, which was activated antidromically at 0.9 msec (Figure A), VL stimulation induced a depolarizing potential change, presumably excitatory postsynaptic potential (EPSP), with a latency of 1.4 msec (B). The EPSP had a relatively simple configuration similar to that observed in cat's motoneurones[12]. The small value of the latency suggests that the EPSP was induced monosynaptically or through at the most two synapses. As seen in Figure C–E, when two stimuli were applied to VL successively at various intervals, the respective EPSP's summated on each other without appreciable alteration in their size (C) except for the very short interval of 1.0 (D)–0.6 (E) msec where the axonal conduction fails due to refractoriness. The lack of the temporal facilitation would thus be indicative that the VL-evoked EPSP was monosynaptic in origin.

In 40 out of 70 PTN's so far examined, the latency to the antidromic spike was so short, ranging from 0.7–1.9 msec, that they could be grouped as fast PTN's. The latency of the VL-evoked EPSP's in them was uniformly short, from 1.4–1.7 msec (mean 1.54 msec). On the other hand, the remaining 30 PTN's belonged to the slow PTN's, for the antidromic latency was as long as 2.3–5.2 msec. In these PTN's, as exemplified in Figure G for a cell with the antidromic latency of 2.9 msec (F), EPSP's were induced from VL only with latency as long as 12 msec and their amplitude as well as their latency fluctuated much during repeated stimulation. The latency of the EPSP's varied over a wide range, from 2.2–20 msec, even though the minimum value was taken in each case. No EPSP was seen in the other 9 cells of the slow PTN's. These results suggest that the slow PTN's were activated polysynaptically by VL stimulation.



Intracellularly recorded potentials of PTN's. (A) antidromic spike potential recorded from a fast PTN. Pyramid stimulation was set just at threshold intensity; (B) EPSP's obtained by VL stimulation in the cell of A; (C–E) double shock to VL; (F) antidromic spike of a slow PTN; (G) EPSP's evoked by VL stimulation in the cell of F; (H) EPSP evoked by double shock to BC; (I) showing that single shock to BC, of the same intensity as in H, had no effect. All records are formed by superimposition of about 30 faint traces. Positivity is represented by upward deflection. Time scale is 1 msec. Voltage scale of 5 mV in E applies to B–E and that of 10 mV in I to H–I.

BC was stimulated in 15 out of 40 fast PTN's. In all of them, EPSP's were elicited at latencies of 2.7–3.5 msec and it showed also a simple configuration (H). The two successive stimuli applied at an interval of 1.5 msec to BC produced the EPSP (H), even though the intensity of either stimulus was subthreshold for inducing any potential changes (I). This temporal facilitation indicates that the pathway for the BC-evoked EPSP in the fast PTN's should be mediated through at least one synaptic relay. On the other hand, the intracellular recording from the VL neurones revealed that the monosynaptic EPSP was elicited by BC stimulation in them with the latency of 1.5 msec[13]. So the latency of the BC-evoked EPSP in the PTN's could reasonably be accounted for by the sum of the latency of the BC-evoked EPSP in the VL neurones, some delay time (0.5 msec or so) for excitation of VL cells by that EPSP, and the latency of the VL-evoked EPSP in the PTN's. Hence it is likely that the pathway responsible for the BC-evoked EPSP in the PTN's is disynaptic, with one relay at the VL neurones.

It is concluded that the cerebello-thalamocortical excitatory action is exerted primarily upon the fast PTN's, there being only subsidiary influences on the slow PTN's.

Résumé. L'activité électrique des neurones du faisceau pyramidal est étudiée ici chez des chats anesthésiés au Nembutal au moyen d'un enregistrement intracellulaire. La stimulation du noyau ventral latéral thalamique induit un potentiel postsynaptique excitateur (EPSP) monosynaptique et la stimulation du brachium conjunctivum induit un EPSP disynaptique dans les neurones pyramidaux de conductibilité rapide.

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