Periodic fluctuation in burst amplitude during 'fictive locomotion' in the cat

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Summary. In decorticated unanaesthetized and paralysed cats, locomotor bursting may develop spontaneously on hindlimb motor nerves. In defined experimental conditions, the amplitude of successive rhythmic bursts may fluctuate periodically as if a 'secondary oscillation' was superimposed upon the basic locomotor rhythm. The possible meaning of such amplitude fluctuation is discussed in connection with present data about the generation of locomotion.

It is now quite clear in both invertebrates and vertebrates that the programme underlying locomotor behaviour lies entirely in the central nervous system[1-6]. Locomotor rhythms are assumed to be generated by central nervous oscillators without the help of proprioceptive or exteroceptive feedback. Thus, for example, in decorticated unanaesthetized and paralysed rabbits and cats, spontaneous sequences of rhythmic locomotor bursts ('fictive locomotion') may be recorded on motor nerves of the limb muscles. The present study establishes that, in defined experimental conditions, such discharges are not identical one with the other, i.e. their amplitude may fluctuate periodically as if a 'secondary oscillation' was superimposed upon the basic primary locomotor rhythm.

Methods. Experiments were performed on unanaesthetized adult cats paralysed with gallamine (Flaxedil). During a surgical procedure carried out under halothane anaesthesia the animals were decorticated and hindlimb motor nerves to the tibialis anterior (TA, ankle flexor muscle) and the gastrocnemius medialis (GM, ankle extensor) were isolated from their central cut ends for recording. Both hindlimbs were placed in a standard symmetrical half-flexed position so that the tonic afferent inflow due to the limb posture could not influence the relative importance of flexor and extensor nerve activities[7,8]. Heart rate, blood pressure and end-tidal CO_2 were constantly monitored and the level of curarization was maintained as constant as possible (6 mg/kg/h) at supramaximal doses blocking both extra- and intrafusal motor innervation.

Results. As previously described in the decorticated and paralysed preparation[2,9,10], spontaneous locomotor bursts recorded on hindlimb muscle nerves develop alternately on antagonistic TA and GM nerves according to the reciprocal innervation law (figure, I). Their frequency may fluctuate from 0.1 to 2 Hz and their duration from 0.2 to 3 sec depending on the animal's 'nervous excitation level' which can undergo considerable changes with time in this type of decorticated preparation. Successive rhythmic discharges are grouped in locomotor sequences which may be separated from each other by silent intervals lasting for periods from a few sec to several min. However, when the 'excitation level' of the animal remains stable for a good while (i.e. when spontaneous rhythmic bursts regularly follow one another with weak variations in their duration), we may observe inside each sequence, both on TA and GM nerves, a progressive increase in the amplitude of successive bursts followed by a progressive decrease (figure, I). So, if locomotor sequences are close enough to one another, such fluctuations thus seem like a sort of 'secondary oscillation' superimposed upon the basic primary locomotor rhythm (figure, II). This phenomenon cannot have a defined period for the following main reasons: a) neither the number of bursts inside a sequence nor the time interval between sequences is constant (figure); b) the discharges with maximum amplitude (which can reach 100%) do not necessarily take place in the middles of consecutive sequences, and they can shift towards the beginning or the end of a sequence (figure, II). Otherwise, no correlation could be established between the value of the peak amplitude and the burst frequency inside a sequence, nor with the time duration of the sequence.

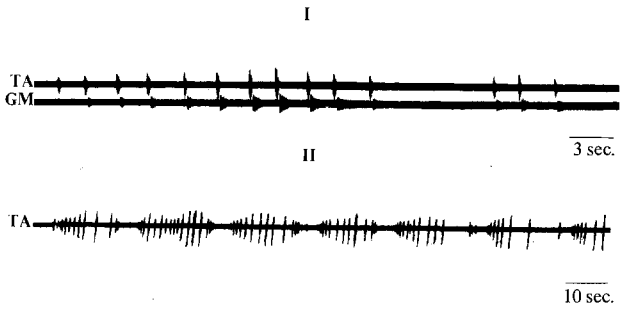
We must state that such periodic amplitude fluctuation, which is not an artefact according to the preceding remarks, is quite obvious when the recording speed of locomotor discharges is slow enough, i.e. about 1-2.5 mm/sec.

Discussion. The present data call for several remarks and comments: 1. In multifiber recordings, such as in our experiments, the amplitude of locomotor bursts is proportional to the number of active motor fibres as well as to their firing rate[11]. It would be interesting to know whether both or only 1 of these 2 parameters is involved in the variation observed in the burst amplitude.

2. We have noticed that the amplitude fluctuation is concomitant on antagonistic muscle nerves TA and GM and occurs in a similar way on both. It may be supposed that such an oscillatory process is first exerted at the level of the rhythm generator itself, and then alternately transmitted to flexor and extensor motoneuronal pools. Otherwise, a system proper to each motoneuronal pool which may underlie self-activation and inhibition must be identical for flexor and extensor motor nucleus. Moreover, it has been pointed out that the value of the maximum amplitude is independent of the burst frequency; it thus seems that the process of rhythm regulation is not coupled with the process which controls the firing rate and the recruitment of motoneurones.

3. Another point of discussion concerns the nervous structures involved in the generation of the amplitude oscillation. Is it a phenomenon inherent in the decorticated preparation, i.e. with supraspinal structures involved, or is the spinal cord sufficient for its generation? It would be particularly worth knowing whether the secondary oscillation could be found again in the different pathways and nuclei brought into play with locomotion, especially the ventral spino-cerebellar tract (VSCT) and also the reticular, rubral and vestibular nuclei.

4. The question of a possible modulation of the amplitude of oscillation by peripheral and central influences has to be



Examples of periodic fluctuation in the amplitude of spontaneous locomotor bursts recorded on hindlimb antagonistic muscle nerves TA (ankle flexor) and GM (ankle extensor). **I** Simultaneous recordings on nerves to TA and GM: the 1st locomotor sequence (with 11 bursts) as well as the following one (with only 3 bursts) show periodic oscillations in their burst amplitude. **II** Series of several consecutive sequences of locomotor discharges where the 'secondary oscillation' becomes quite visible.

raised. In particular, the effects of stimulation of cutaneous or proprioceptive afferents as well as monoaminergic descending pathways could be studied since they are known to elicit activation and modulation of locomotor activities[5,7,8,11-15]. Such studies are now in progress as well as a detailed quantification of the results.

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Diabetogenic action of alloxan-like derivatives of uric acid

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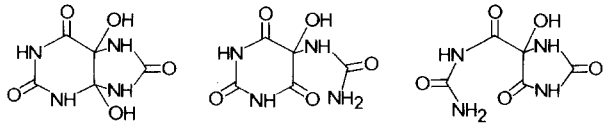
Summary. Two new potent diabetogenic substances 4,5-dihydro-4,5-dihydroxyuric acid (**1**) and 5-hydroxy-pseudouric acid (**2**) have been found.

The production of experimental diabetes by alloxan led to the early suggestion that in human diabetes an alloxan-like metabolite is formed in the course of purine catabolism[2]. Attempts to detect alloxan in vivo[3,4] and to demonstrate that it can be derived from uric acid[5,6] have also been reported. Our recent re-examination of the chemical and spectroscopical properties of alloxan-like compounds **1–3**, have resulted in the revision of previously accepted structures[7,8].



1 2 3

The structures **1** and **2** were assigned to Biltz' 5-hydroxy-pseudouric acid[9] and Behrend's compound $C_5H_6N_4O_5$[10], respectively. The structure **3** was established for the 3rd isomeric compound, originally formulated as 4,5-dihydro-4,5-dihydroxyuric acid[11].

An investigation of the biological activity of alloxan-like derivatives of uric acid seemed necessary in view of its far reaching importance in understanding the pathogenesis of diabetes mellitus.

Materials and methods. Male Lewis rats, weighing 180–230 g, were used throughout. The substances tested were injected i.v. or i.p. as saline solutions or suspensions, depending on solubility. A rat was considered strongly diabetic if glucosuria (> 1%) occurred within 24 h after injection and persisted for 5 days. Additional information was obtained by observation of blood sugar, ketonuria, and histological changes. The diabetogenic potency of the substance was expressed as the effective dose which caused diabetes in 50% of the animals (ED_{50})[12].

Results and discussion. The substances **1–3** were tested for diabetogenic activity. I.v. administration of **1** and **3** was inconvenient due to low solubility, therefore these substances were injected i.p. For comparison, activity of alloxan was also tested and the results are summarized in the following table.

A single i.p. or i.v. administration of **1** or **2** caused diabetes, whereas **3** produced neither diabetes nor toxic symptoms in rats. Nevertheless a reliable comparison is doubtful on account of remarkable differences in solubility; activities of **1** and alloxan, administrated i.p., were similar. The alloxan content at ED_{50} of **2**, however, corresponded to only 24 mg/kg (0.15 mmoles/kg), an amount which does not in itself cause diabetes when given i.v. Since the lowest observed active dose of alloxan is 30 mg/kg (0.19 mmoles/kg), there can be no doubt that the active species is the ureide **2**. Severe cases of diabetes with extreme hyperglycemia, ketonuria, and heavy glucosuria first began to appear in all animals injected with 50 mg/kg of the more potent diabetogenic substance **2**, although it seemed to be less toxic than alloxan. The permanent hyperglycemia was produced within 24 h after administration of the diabetogenic dose of **1** or **2**, and no typical triphasic change in the blood sugar curve as observed in alloxan diabetes was noted. The histological changes in the islets of Langerhans were similar to those in alloxan diabetes. Compounds **1** and **2** caused selective injury to the β-cells, resulting in the reduction of islets which consisted almost entirely of α-cells. The metabolic implications of these findings are of some interest. It seems likely that under certain conditions in vivo uric acid can be converted to intermediates **1** and/or **2** and thus explain the lesion of β-cells of the islands of Langerhans produced as the result

Effective diabetogenic doses (ED_{50}) in rats

Substance	No. of rats injected	(ED_{50}) mg/kg b.wt	mmole/kg b.wt
Alloxan	16 (i.v.)	50	0.31
Alloxan	16 (i.p.)	180	1.12
1	16 (i.p.)	215	1.06
2	24 (i.v.)	30	0.15
3	8 (i.p.)	inactive with 4 g/kg	