On the Action of 3-Hydroxytyramine and Dichloroisopropylnoradrenaline on Spinal Reflexes

In a recent paper the effect of the topical application of 3-hydroxytyramine to the exposed spinal cord upon the knee jerk reflex in cats was described[1]. Strong solutions (2.5–5% w/v) of this catecholamine brought about the inhibition of the reflex muscle contractions in response to tapping the patellar tendon, whereas equivalent concentrations of adrenaline or of noradrenaline were largely ineffective. This action of 3-hydroxytyramine, and the inhibition of the knee jerk brought about by stimulation of the bulbar reticular formation[2], could be prevented by the administration of dichloroisopropylnoradrenaline (DCI) to the animal.

Two possibilities appear to exist to explain these results: either (a) 3-hydroxytyramine mimics the action of stimulation of inhibitory interneurones acting upon the motoneurones (i.e. it is active upon the subsynaptic patches at inhibitory synapses), or (b) it acts to excite inhibitory interneurones whose excitation in turn results in inhibition of the motoneurones. If (a) were true, then DCI might be expected, since it blocked the action of topically applied 3-hydroxytyramine, similarly to reduce the action of a physiological inhibitory pathway, of which the simplest is the 'direct' inhibition of LLOYD[3]. If (b) were the case, some indication of enhanced interneuronal discharge should be observed in the cord under the influence of topically applied 3-hydroxytyramine.

Experiments were performed upon cats anesthetized with chloralose (65 mg/kg) and urethane (250 mg/kg). Figure 1A shows the time course of the direct inhibition of a monosynaptic reflex arc set up by stimulation of the lateral gastrocnemius nerve, inhibitory stimuli being applied to the deep peroneal nerve[3]. Records were obtained from a ventral root filament. It is evident that after the administration of DCI in a dose of 7 mg/kg i.v., the inhibition of the reflex was completely unaffected. This dose of DCI was sufficient, however, largely to suppress the inhibition of the reflex brought about by stimulation of the reticular formation[1,2] as is shown in Figure 1B. It was concluded that the blocking action of DCI upon this latter inhibitory process is not exerted at the level of the motoneurone. It seemed likely that alternative (a) is therefore untenable.

Although the inhibitory Renshaw interneurones of the anterior horn of the cord are known to receive cholinergic innervation[4], and therefore might not be expected to be excited by 3-hydroxytyramine, their characteristic discharge pattern has been observed under the influence of the substance topically applied to the cord surface. Records were obtained with the aid of a microelectrode filled with 4 *M* NaCl inserted into the cord, while antidromic stimulation leading to excitation of the Renshaw cells was applied to the ventral roots. The firing pattern of Renshaw cells was found to be unaffected by the application to the cord of 5% 3-hydroxytyramine. Furthermore the occasional 'spontaneous' discharge of the cells was not influenced by the application.

In other experiments records have been obtained of the discharges of single cells or of small groups of neurones in or near the intermediate nucleus of Cajal, in which there lie interneurones both of excitatory and inhibitory reflex function[5]. Such cells have been excited by stimuli applied to various muscle nerves, or to those areas of the reticular formation giving inhibition of reflexes in the cord. The behaviour of these interneurones to the application of 3-hydroxytyramine has been found to be variable. The majority have been completely unaffected. Others, particularly those responding to reticular formation stimulation, have shown an increased excitability as determined from their lowered threshold for activation, or, as in Figure 2, an enhanced discharge of a group of cells subjected to a submaximal stimulus.

CURTIS[6] has recently described experiments in which 3-hydroxytyramine was applied iontophoretically to

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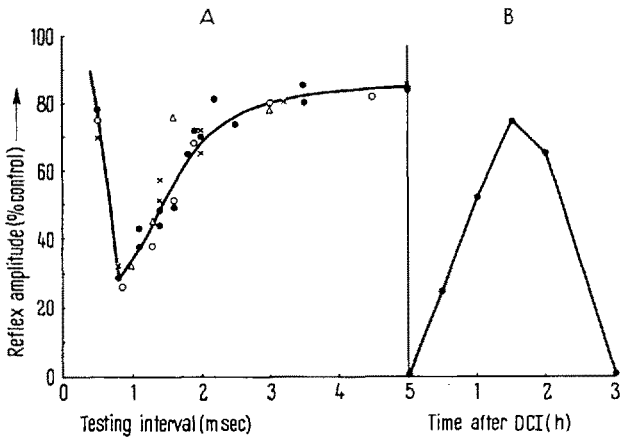

Fig. 1. A Time course of the 'direct' inhibition of a monosynaptic reflex in gastrocnemius motoneurones by preceding impulses at varying intervals in the deep peroneal nerve. Controls (•); (×) 30 min, (o) 1 h, (△) 1¾ h after the i.v. administration of DCI, 7 mg/kg. B The effect of DCI in the same animal, upon the inhibition of the reflex brought about by stimulation of the bulbar reticular formation. The points represent the amplitude of the reflex response during inhibitory stimulation as a percentage of the uninhibited response.

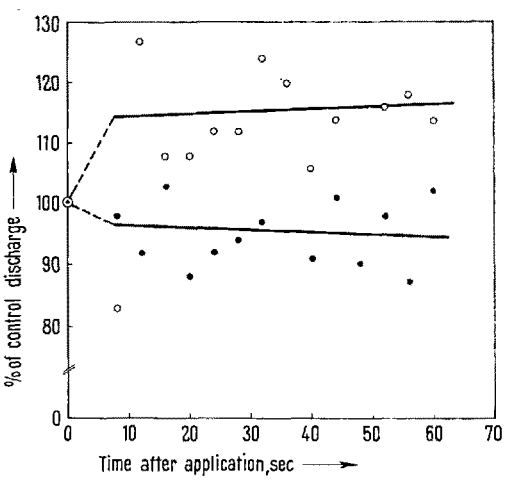

Fig. 2. The integrated discharges of small groups of spinal interneurones, following application to the cord of a 5% solution of 3-hydroxytyramine. (o) From a group fired by stimulation of the reticular formation, whose discharge was augmented by the application. (•) From a group fired by stimulation of the lateral gastrocnemius nerve, which was unaffected by the same application.

various types of spinal cord neurones, and has found it not to affect interneurones, Renshaw cells or motoneurones. The latter two observations are in accord with the results presented here, and it is possible that those interneurones studied by CURTIS were all of the type which have been found to be uninfluenced in the present experiments also. The results which have been obtained here would suggest that a possible explanation of the action of applied 3-hydroxytyramine reported earlier[1], is that it leads to the excitation of inhibitory interneurones which make synaptic contact with the motoneurones of the cord.

Zusammenfassung. «Direkte» Hemmung monosynaptischer spinaler Reflexe in Katzen wird durch Administration von DCI nicht verhindert, obwohl die Hemmung nach Reizung der bulbären retikulären Formation ausbleibt. 3-Hydroxytyramine, dessen reflexhemmende Wirkung bei direkter Applikation am entblössten Rückenmark früher gezeigt wurde, verursacht eine gesteigerte Erregbarkeit mancher Interneurone der retikulospinalen Leitungsbahn, wodurch seine hemmende Wirkung erklärt werden könnte.

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Antidiuretic Hormone Concentration in Blood Perfusing the Adenohypophysis

The concept of the antidiuretic hormone (ADH, vasopressin) as a physiological stimulus for adenohypophysial ACTH secretion has been questioned recently[1-4]. An argument often employed against this once attractive hypothesis is based upon the fact that the concentration of injected Pitressin or vasopressin which stimulates ACTH secretion is much larger than that mimicking physiological antidiuresis. Because a high concentration is not normally encountered in peripheral blood, this action of vasopressin on the adenohypophysis is interpreted as pharmacologic. Implicit in this interpretation is the assumption that high adenohypophysial and low peripheral blood concentrations of ADH are physiologically mutually exclusive.

The purpose of this paper is to demonstrate that both conditions do, in fact, normally exist. Our reasoning follows and is based on a calculation of the ADH content in blood leaving the neural lobe and directly perfusing a major portion of the adenohypophysis. The data used in our calculations are our own measurements of neural lobe blood flow[5], coupled with GINSBURG's estimate of the half-life of circulating ADH[6], HELLER's measurements of the resting ADH level in the rat[7], and DE WIED's data for the ADH response to haemorrhage in the rat[8].

The resting level of ADH in the adult albino rat is 2.3 μU/ml whole blood[7]. A 300 g rat with about 15 ml blood volume therefore has a total circulating ADH content of about 35 μU. According to GINSBURG[6] the half-life of circulating ADH in a rat under similar conditions is about 42 sec. Assuming that the neural lobe is the only source of ADH, this means that this gland must add 2.5×10^{-5} U to blood perfusing it each minute. If the blood flow fraction through the neural lobe is 5×10^{-5} times the cardiac output, which is about 90 ml/min in our unanesthetized rats, the blood flow through the gland is about 4.5×10^{-3} ml/min[5]. The concentration of ADH in the neural lobe effluent of the resting rat must therefore exceed that in the arterial input by

$$2.5 \times 10^{-5} \text{ U/min}/(4.5 \times 10^{-3} \text{ ml/min}) = 5.6 \times 10^{-3} \text{ U/ml}.$$

During the last 20 sec of a substantial haemorrhage (5 ml in 90 sec) DE WIED's data indicate that the change in peripheral concentration of ADH is approximately 3×10^{-2} U/2×10^{2} ml whole blood/20 sec or 4.5×10^{-4} U/ml whole blood/min[8]. Repeating the earlier calculations, the amount of ADH added to the general circulation under these circumstances must be $15 \text{ ml} \times 4.5 \times 10^{-4}$ U/ml/min or 6.75×10^{-3} U/min. Since the neural lobe blood flow is 4.5×10^{-3} ml/min the concentration of ADH in this blood must exceed that in the arterial input by

$$(6.75 \times 10^{-3} \text{ U/min})/(4.5 \times 10^{-3} \text{ ml/min}) = 1.5 \text{ U/ml}.$$

Correction for the half-life of ADH in the general circulation would raise this value by some 25%, or close to 2 U/ml. This represents a 300 fold increase in ADH output over the resting level; and, since ADH can be suppressed below this level, the dynamic range of the ADH secreting mechanism should be in excess of 300.

The adenohypophysis has no (or almost no) direct arterial supply, instead receiving its vascular input from a group of elegantly controllable parallel portal systems draining parts of the neurohypophysis[9-12]. That a substantial fraction of the adenohypophysis receives its input from the neural lobe has also been established[13]. Since it is reasonable to expect all neural lobe effluent—whether portal or systemic—to carry the same concentration of ADH, this fraction of the adenohypophysis must indeed 'see' a very high ADH concentration, which is subsequently diluted more than 20000 times in the general circulation.

In view of our calculations, interpretations suggesting a pharmacologic (as opposed to physiologic) action of vasopressin on ACTH secretion which are based primarily on responses to systemic injections must be reevaluated. The phenomena occurring between the neurohypophysis and adenohypophysis are local and not reproducible by events occurring in the general circulation. In order to achieve, by peripheral intravenous injection, levels of local vasopressin or Pitressin concentration equivalent to those here calculated it would be necessary to administer heroic doses: in a 300 g rat, 80 mU for the basal state, 28 Units for extreme stress. The first dose is well within the range

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