luminal fluid initially, was calculated in µl/g wet wt for 45 min. There were 5 rats in each group. The results of these experiments are given in the figure.

*Discussion.* The results showed a net mean (±SEM) absorption of fluid in the saline control of $-308 \pm 89.2$ and in control pre-treated with indomethacin $-309.6 \pm 37.1$ (groups 1 and 2). Experimental animals receiving VIP (group 3) secreted fluid into the gut $255 \pm 114.9$ but this effect was completely reversed $-292 \pm 66.3$ in the final group receiving indomethacin and VIP (group 4). Unpaired 2-tailed Student's test showed highly significant difference between groups 3 and 4 ($t = 4.14$, $p < 0.005$). The model demonstrates that in vivo parenteral porcine VIP produces a net secretion of fluid into the small bowel of rat (probably by increasing jejunal secretion and decreasing ileal absorption). Indomethacin 5 mg/kg has no direct effect on the net absorption normally at rest but when given 15 min before the infusion of VIP it completely inhibits the onset of net secretion. In other studies longer term and more localised in vivo studies on rat jejunum, indomethacin alone has been shown to produce a slight decrease in secretion[9].

Despite this effect implying the direct involvement of prostaglandins, there is little corroborative evidence. For example, VIP has not been able to release prostaglandins in 2 in vitro systems studied: guinea-pig lung perfusion and platelet aggregation[19] (unpublished observation). There is dissociation between hyperprostaglandinemia and circulating VIP in the WDHA syndrome[20], while during cholera enterotoxin-induced secretion VIP rather than prostaglandin-like substances are released from the mucosa[21,22]. Finally there is evidence against indomethacin operating solely through inhibition of prostaglandin synthesis as in vitro studies at concentrations high enough to inhibit synthesis did not prevent the accumulation of cAMP[9,23].

The mechanism whereby indomethacin inhibits cholera enterotoxin and now VIP-stimulated secretion thus remains somewhat obscure. Further work with this model and direct measurement of prostaglandin concentrations, adenyl cyclase activity and intracellular cAMP together with ion fluxes may be able to resolve the difficulties.

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## A quantitative study of the catecholamine-fluorescence in the ganglion paracervicale uteri of the rat

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*Summary.* In the ganglion paracervicale uteri of the rat, there are principle neurons which are able to take up offered catecholamines. Normally there is an inverse relationship between their size and their mean catecholamine-fluorescence. A comparison with the catecholamine-fluorescence of depleted and repleted adrenergic perikarya in the ganglion cervicale superius is made.

The paracervical ganglion of the rat is said to consist of cholinergic perikarya, small, intensely fluorescent cells (SIF-cells), and adrenergic perikarya which are responsible for the innervation of at least part of the female genital tract[2,3]. Whereas there is no dispute about the existence of the SIF-cells and cholinergic perikarya in this ganglion, Baker et al.[4] doubted the existence of paracervical principle neurons, as they showed, there was no dopamine beta-hydroxylase, a noradrenaline synthesizing enzyme[5], in these cells.

The aim of this study was to reconsider the adrenergic nature of these principle neurons by testing their ability to take up offered catecholamines and to release catecholamines after treatment with reserpine. Changes in cellular catecholamine content were estimated by means of formaldehyde induced fluorescence and compared to those in the superior cervical and nodose ganglia.

*Methods.* a) Animals. 16 female Sprague-Dawley derived albino rats weighing 200–220 g were housed in cages under conditions of constant light:dark cycles (12 h), constant humidity and temperature with food and water ad libitum. They were divided into 4 groups:

group C: 4 controls, no treatment, excised ganglia treated with formaldehyde gas;

group U: 2 controls, no treatment, excised ganglia not treated with formaldehyde gas;

group R: 5 animals treated with reserpine 10 mg/kg b.wt i.p., 24 h before excision of samples;

group D: 5 animals treated with reserpine 10 mg/kg b.wt i.p. 24 h + nialamide 1000 mg/kg b.wt i.p. 2 h + L-DOPA-methylester 7.5 mg/kg b.wt i.p. 20 min before excision of samples.

The animals were sacrificed in ether anaesthesia by decapitation and the right superior cervical and nodose ganglia as

well as the right or left paracervical ganglion were immediately excised.

b) Histochemical procedure. The formaldehyde-induced fluorescence method of Falck and Hillarp[6] was used: The excised samples were directly put in liquid nitrogen, freeze-dried, and treated with formaldehyde gas at 80 °C for 75 min. The gas was generated from paraformaldehyde powder equilibrated at 50% relative humidity. The samples were embedded in paraffin and sections (5-µm-thick) were examined microfluorimetrically. The method for the evaluation of the intensity of fluorescence and for the determination of the length and width of the observed perikarya was the same as that described by Krinke et al.[7]. By purpose, no SIF-cells were measured.

In the ganglion cervicale superius (GCS), the size and intensity of fluorescence of 80–100 perikarya were determined in each group. The corresponding numbers for the ganglion nodosum (GN) and ganglion paracervicale (GPC) were 51–53 and 71–73, respectively.

c) Statistics. For each ganglion, the mean fluorescence intensity/perikaryon was computed and expressed in arbitrary units (AU). From this, the average fluorescence intensity (IF) of the whole experimental group was determined. In order to eliminate as far as possible errors due to the autofluorescence of tissue, the mean IF of group U was subtracted from this value. Differences among particular experimental groups were assessed by means of the analysis of variance.

*Results.* The IF of the principle neurons in the superior cervical ganglion was lower in groups R and D than in the controls. The average IF of group D was higher than that of group R, both in the superior cervical and the paracervical ganglion (table 1, figure 1).

A comparison of the IF of the ganglia within each group is shown in table 2 (Student's t-test).

For each group, the perikarya of a ganglion were classified according to the size of their sectional-plane area and their average IF were computed. Thus a significant inverse relationship between their mean IF and their size was found for all groups in the GCS and for group C in the GPC (figure 2). No correlation was found for the perikarya of the ganglion nodosum. To evaluate the effect of reserpine on different classes of sizes of perikarya, their IF of group R was plotted as a function of that of group C. The mean IF of perikarya after reserpinisation was proportional to the mean IF before reserpinisation with decreasing size of perikarya. This relationship could be found for the GCS only (table 3).

*Discussion.* In control rats, the mean IF of the GCS perikarya is higher than that in the GPC, and the average IF of the latter ganglion does not significantly differ from that in the nodose ganglion, which is not adrenergic. From this it could be inferred that the paracervical ganglion in

Table 1. Comparison of intensity of fluorescence (IF) of principle neurons within each ganglion; GCS = ganglion cervicale superius, GN = ganglion nodosum, GPC = ganglion paracervicale (analysis of variance)

| Ganglion | Comparison of IF | p |
|---|---|---|
| GCS | R < C | < 0.001 |
| | D > R | < 0.05 |
| | C > D | < 0.001 |
| GN | R C | n.s. |
| | D R | n.s. |
| | C D | n.s. |
| GPC | R C | n.s. |
| | D > R | < 0.05 |
| | C D | n.s. |

Table 2. Comparison of the IF of the ganglia within each group

| Group | Comparison of IF | p |
|---|---|---|
| C | GCS > GN | < 0.001 |
| | GCS > GPC | < 0.001 |
| | GN GPC | n.s. |
| R | GCS GN | n.s. |
| | GCS GPC | n.s. |
| | GN GPC | n.s. |
| D | GCS > GN | < 0.001 |
| | GCS GPC | n.s. |
| | GN GPC | n.s. |

Table 3. Ganglion cervicale superius: Average of fluorescence intensity (IF) of classes of perikarya before (x) and after (y) reserpinisation. Values of IF in arbitrary units (AU)

| Class of sectional plane area (µm²) | Average IF (AU) Before reserpinisation (x) | After reserpinisation (y) |
|---|---|---|
| 376–450 | 1086.53 | 414.50 |
| 451–525 | 895.13 | 343.00 |
| 526–600 | 802.20 | 352.82 |
| 601–675 | 698.63 | 264.14 |
| 676–700 | 637.00 | 292.00 |

Correlation: $y = 0.303\,x + 93.278$, $r = 0.922$, $p < 0.05$.

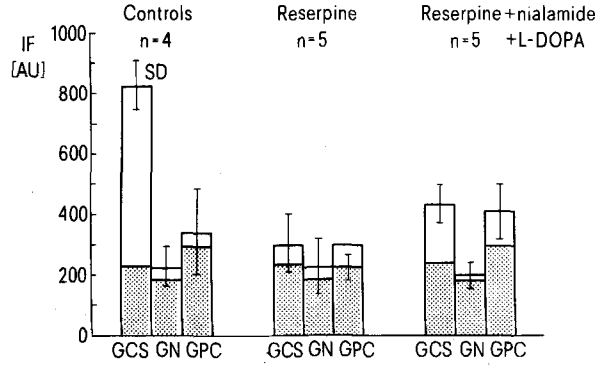


Fig. 1. Mean fluorescence intensity (IF) of principle neurons expressed in arbitrary units (AU) ± SD in the superior cervical (GCS), nodose (GN) and paracervical (GPC) ganglia of groups C, R and D. Hatched areas: mean values for samples not treated with formaldehyde gas.

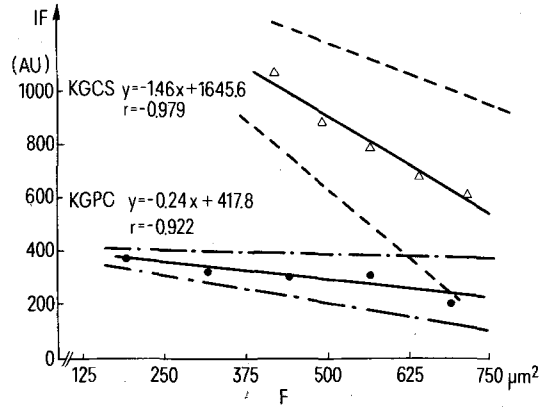


Fig. 2. Correlation of fluorescence intensity (IF) of control principle neurons to their size (sectional plane area) in the GCS compared with the same correlation in the GPC.

fact does not contain principle adrenergic neurons. Regarding the catecholamine-uptake after application of L-DOPA, however, there is a significant difference between the nodose and the paracervical ganglion, as in the GPC there are cells able to take up catecholamine precursors, and these are either adrenergic neurons or they belong to the APUD system (SIF-cells in our case)[8]. Since in our study SIF-cells were purposely omitted, the cells capable of uptake were most likely adrenergic perikarya. In the GCS, there was a considerable decrease of IF after treatment with reserpine, while the mean IF of the GPC after reserpinisation did not differ significantly from that of the controls. This does not mean that the perikarya in the GPC as short neurons are more resistent to reserpine[9]; rather, the endogeneous catecholamine content in the GPC is very low.

In the superior cervical ganglion, there is an inverse relationship between the size of the perikarya and their mean IF[7]. In our study, the same relationship was observed, and in addition it also was found for partly depleted and repleted principle neurons of this ganglion, as well as for the control perikarya in the paracervical ganglia in spite of their low endogeneous IF. We suggest that this relationship is typical for adrenergic principle neurons, whether they belong to long or to short adrenergic neurons.

In figure 2 it is evident that the total amount of catecholamines in the GCS is higher than that in the GPC. The GCS is responsible for the innervation of metabolically very active organs like those of the head and part of the heart[10], whereas the GPC innervates comparatively inert organs like uterus, tube and ovary. It is well known that the catecholamine-contents of an adrenergic perikaryon is correlated to its nerve activity[11] which might possibly explain the low catecholamine-contents in the GPC. In the GCS there is a constant relationship between the IF before and after reserpinisation with decreasing size of the perikarya (table 3). This means that the larger perikarya release the same percentual share of catecholamines as the smaller ones upon systemic application of reserpine. Therefore, it can be assumed that reserpine distributes within the adrenergic perikarya in similar way as the endogeneous catecholamines. This relationship could not be found in the GPC, obviously because the initial values were too low and did not differ significantly from those of the reserpinised animals.

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## Lymph node metastases of EMT6 tumour in nude mice

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*Summary.* Metastatic axillary lymph nodes following the injection of EMT6 tumour cells were observed in athymic nude mice, more often in female animals, and had a rapid growth rate. These metastases did not develop in syngeneic hosts. The latency of their appearance was inversely related to the number of injected cells.

It is well known that the prognosis of many types of human cancer is largely dependent on the presence of lymph node metastasis (LNM). It is thus justified to resort to experimental models in order the better to understand this type of spread[2]. The available models dealing with true LNM in mice usually consist of inoculating tumour cells in the foot pad[3-6]; intratibial[7], intratesticular[8], tail[9], thigh[5] and s.c.[10] injections have also been used. To our knowledge, none of these allowed a comparison of the characteristics of the initial tumour with those of LNM. Here we describe the occurrence of LNM in athymic nude mice after s.c. EMT6 cell injection with reference to sex differences and growth rate differences in relation to the primary tumour.

*Materials and methods.* The culture conditions of the EMT6 cell line and the technique of obtaining single cell suspensions have been described elsewhere[11]. Breeding and maintenance of athymic nude mice have also been previously reported[12]. From $1.3 \times 10^2$ to $7 \times 10^5$ cells in 0.1 ml were injected s.c. in the flank region of these mice. $7 \times 10^5$ EMT6 cells were also injected into female syngeneic hosts (BALB/c mice) as a control group.

*Results.* 5 days after the injection of $7 \times 10^5$ cells, a s.c. nodule became palpable having a doubling time (DT) of 2.5 days. This DT became longer as the tumour progressed in size, to reach 20 days before the death of the animals (figure 1). Homolateral axillary LNM started to appear at the beginning of the 5th week (figures 1 and 2) and had a DT of 1.1 day all through. The metastatic character of these

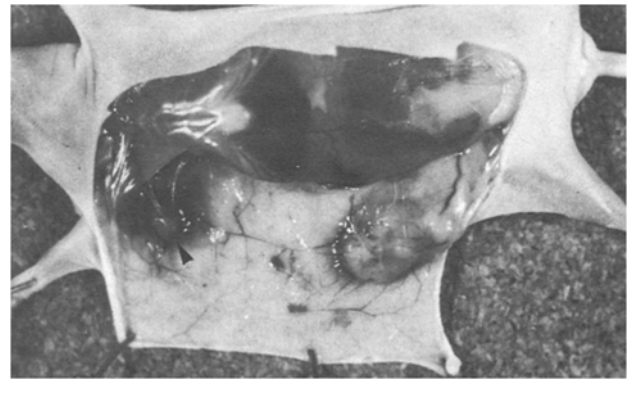

Fig. 1. Growth curves of EMT6 tumour in the s.c. region ○——○, and in the lymph node ●——●, following the injection of $7 \times 10^5$ cells.