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^{3}H-Nicotine in cat superior cervical and nodose ganglia after close-arterial injection *in vivo*

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1. Concentrations of ^{3}H-nicotine in the superior cervical and nodose ganglia of anaesthetized cats were measured after close-arterial injection.

2. Shortly after injection there was a higher concentration of ^{3}H-nicotine in the superior cervical ganglion than in the nodose ganglion. Mean concentration ratios, superior cervical ganglion/nodose ganglion (S/N ratios) were: 2 min after injection, 1.60 ± 0.19 ; 4 min, 1.21 ± 0.19 ; 8 min, 0.92 ± 0.05. These ratios were independent of the dose of nicotine over the range 4 to 200 μg in 0.2 ml.

3. There was no comparable difference in the concentrations of injected ^{14}C-inulin or ^3H_2O in the two ganglia, or in total water content.

4. Procedures which reduced the pharmacological action of nicotine (pretreatment with hexamethonium, admixture of ^{14}C-inulin) tended to reduce the S/N ratio for nicotine.

5. Autoradiographs showed that nicotine entered the neurones of both superior cervical and nodose ganglia.

6. It was concluded that the higher concentration of nicotine in the superior cervical ganglion was probably related to its selective pharmacological action at this site, and may have been due to a greater intracellular retention of nicotine.

Appelgren, Hansson & Schmiterlöw (1963) obtained autoradiographic evidence to suggest that, 5 min after the close-arterial injection of ^{14}C-nicotine, there was much more radioactivity in the cat superior cervical ganglion than in the adjacent nodose ganglion.

In the cat these two ganglia are closely associated and receive the same blood supply, so they would be expected to receive the same proportions of injected nicotine. This raises the question whether the different amounts of nicotine retained in these two structures might be related to their different responsiveness to nicotine, for Langley & Dickinson (1889) reported that, whereas nicotine blocks transmission

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through the superior cervical ganglion, it does not affect conduction through the nodose ganglion. This reflects the fact that the nodose ganglion is a sensory ganglion, containing the cell bodies of afferent vagal fibres, and so is devoid of synapses.

The present experiments were undertaken to obtain more information regarding nicotine retention in these two ganglia, by injecting ^{3}H-nicotine close-arterially and measuring both pharmacological responses and ganglionic tritium concentrations. In addition, the intraganglionic distribution of ^{3}H-nicotine has been studied by autoradiography.

Some of the results have been described briefly to the American Society for Pharmacology and Experimental Therapeutics (Brown, Hoffmann & Roth, 1966).

Methods

Cats were anaesthetized with sodium pentobarbitone (35 mg/kg intraperitoneally). The superior cervical and nodose ganglia and their associated nerve trunks were exposed, and the lingual artery cannulated for retrograde close-arterial injection as described previously (Brown & Quilliam, 1964). Drugs were injected through the lingual artery while the external carotid artery was occluded, the injection volume being 0.2 ml. The injections were made rapidly (about 1 sec), so that the blood in the common carotid artery immediately below the ganglion was replaced by a "slug" of injection fluid. This was then forced, in apparently undiluted form, through the ganglion to produce a response within less than 5 sec of beginning the injection. Judged by the rate at which the injection slug in the common carotid artery was replaced by blood, the injection fluid was substantially cleared from the ganglion within 30 sec or thereabouts. Responses of the superior cervical ganglion to drug injections and to preganglionic cervical sympathetic nerve stimulation were monitored by recording the contraction of the corresponding nictitating membrane, either on a smoked drum using a frontal writing lever, or on a multi-channel recorder ("Physiograph," E. and M., Houston, Texas), using a myograph (E. and M.). Conduction through the nodose ganglion was assessed by stimulating the central end of the cut vagus nerve. This produced an inhibition of respiration and a fall of arterial blood pressure, which were recorded on a multi-channel recorder (see Fig. 1). Respiration was recorded using an impedance pneumograph (E. and M.). Arterial blood pressure was recorded from the femoral artery with a linear core pressure transducer (E. and M.). Stimulation parameters were: pulse duration, 0.1 msec; voltage, 3 V (vagus) and 5 V (sympathetic); frequencies, 10 Hz (sympathetic), and 10 Hz and 50 Hz (vagus).

Ganglionic radioactivity measurement

The superior cervical and nodose ganglia were removed simultaneously from the animal at a predetermined time after injection. Their connective tissue capsules were stripped off, the ganglia were rinsed once by immersion in saline for approximately 2 sec to remove surface activity, blotted and frozen in liquid nitrogen. The frozen ganglia were each weighed on a torsion balance in a cold room at $-20°$ C, and crushed under liquid nitrogen to form a pellet of finely-divided particles. The pellet was dissolved in 0.5 ml. of N/5 aqueous sodium hydroxide solution by stirring at room temperature for 24 hr, by which time a clear solution was formed. Aliquots

(0.2 ml.) of this solution were added to 15 ml. of phosphor solution and radioactivity counted in a liquid scintillation spectrometer (Packard 3000 series). The composition of the phosphor solution was: toluene, 700 ml.; methanol, 300 ml.; PPO, 8 g; and POPOP, 0.05 g. Counting efficiency, determined by adding ^{3}H-toluene as internal standard, was 14%.

The recovery of ^{3}H-nicotine added to the tissue blanks and carried through the extraction procedure, was $98.8 \pm 1.0\%$ (mean $\pm$ S.E. of mean of four determinations). Ganglionic tritium was extracted with chloroform and separated by thin-layer chromatography using the method of Hansson, Hoffmann & Schmiterlöw (1964) in order to identify the radioactive materials present. Radioactivity was associated with unchanged nicotine in the same proportion (95%) as that in the injected material (see later). No biological metabolites of nicotine were detected, which accords with the observation of Hansson *et al.* (1964) that nerve tissue does not metabolize nicotine.

Autoradiography

Autoradiographs were prepared by the method of Stumpf & Roth (1966), which minimizes translocation of unbound, soluble compounds.

Excised ganglia were rapidly frozen in liquid propane cooled in liquid nitrogen, and then placed in liquid nitrogen until used. Sections of frozen ganglia were cut at $-50°$ to $-60°$ C at a thickness of 0.7 to 0.8 μ. The frozen sections were freeze-dried without warming by cryosorption (Stumpf & Roth, 1967), then dry-mounted on microscope slides coated with a thin layer (about 1 μ) of liquid NTB-3 (Kodak) nuclear emulsion and previously dried for at least 48 hr over desiccant. After placement of sections, the slides were stored in the dark at $-15°$ C, then developed in Kodak D19 developer (2–5 min) and fixed in acid fixer (4–5 min), washed, stained briefly with toluidine blue and viewed under a Zeiss photomicroscope. The use of liquid histological fixatives, paraffin or other embedding media, adhesives, thawing or other " wet " forms of section-slide attachment, were avoided to prevent movement of the nicotine within the tissue sections after excision of the ganglia. The autoradiographic resolution was about 1 μ (Brown, Stumpf & Roth, 1969).

To check for sublimation of nicotine during freeze-drying ^{3}H-nicotine was pipetted on to pieces of filter paper, and the radioactivity on the filter paper measured before and after placing the filter paper in the freeze-drying apparatus for 24 hr. No sublimation was detected.

Radioactive compounds

The following radioactive compounds were used: ^{3}H-nicotine (uniformly labelled), specific activity 761 and 70 mc/m-mole (two batches, Radiochemical Centre, Amersham, England, supplied through the Nuclear Chicago Corporation); ^{14}C-carboxyl-inulin, specific activity 3.58 mc/g, molecular weight 5000–5500 (New England Nuclear Corporation, Boston, Mass. U.S.A.); ^{14}C-1-(+)-mannitol, 10.4 mc/m-mole (Radiochemical Centre, Amersham); and tritiated water (New England Nuclear Corporation).

^{3}H-nicotine purity

The radiochemical purity of the ^{3}H-nicotine was stated by the suppliers to be 94%. The radiochemical purity of the aqueous solution used for injections was

checked before and during the experiments by thin-layer chromatography using silica-coated glass plates and the following solvent system: ethanol-acetone-benzene-concentrated NH_4OH (proportions by volume, 1 : 8 : 10 : 1, Hansson *et al.*, 1964). Radioactivity on the plate was located by autoradiography using Kodak X-ray film, and was measured by removing the silica from the plate and suspending it directly in phosphor solution with a thixotropic silica powder ("Cab-O-Sil," Packard) or by extracting the radioactivity from the silica with water and adding aliquots of the water to phosphor solution. The radiochemical purity of the first batch (761 mc/m-mole) accorded with the suppliers' specification up to 1 month after making up the aqueous solution, but after 3 months' use (and storage at $-10°$ C) secondary spots were detected on the radiochromatograph amounting to 15.1% of the total radioactivity. This sample was discarded thereafter. The second batch, which was used for most of the experiments described in this paper, showed a radiochemical purity of 96% both before and after the experiments were completed.

Unlabelled compounds

Unlabelled nicotine, hexamethonium chloride, (+)-mannitol and inulin (Fisher Biochemicals) were also used. Doses refer to the weight of salt injected in 0.2 ml. of saline solution.

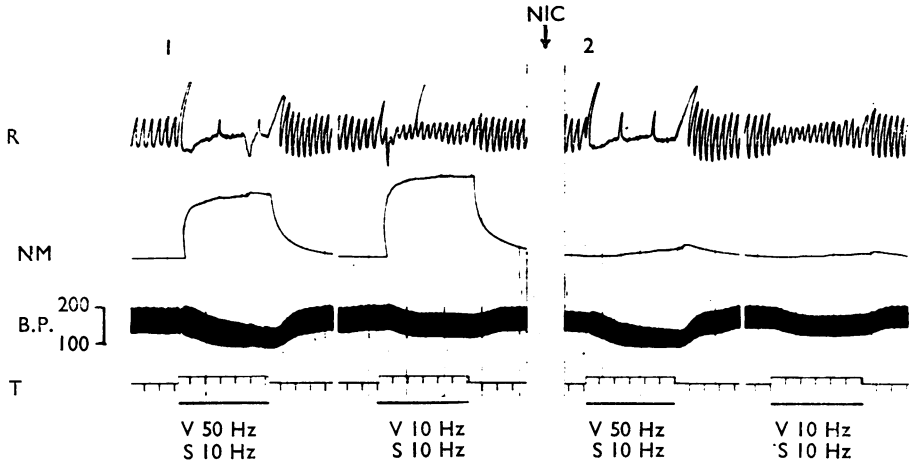

FIG. 1. Differential effects of nicotine on transmission through the superior cervical ganglion and conduction through the nodose ganglion in an anaesthetized cat. The records show: R, respiratory movements (inspiration upwards); N.M., contraction of the nictitating membrane; B.P., arterial blood pressure (scale in mm Hg); T, time in 5 sec intervals. The central end of the cut vagus nerve (V) and the ascending preganglionic cervical sympathetic nerve (S) were stimulated simultaneously for the duration shown by the horizontal bars. The stimulation parameters were: pulse duration, 0.1 msec; voltages, 3 V (vagus) and 5 V (sympathetic); pulse frequencies as shown on the record. Nicotine (NIC, 200 μg/0.2 ml. injection fluid) was injected close-arterially to the two ganglia through the lingual artery at the time shown by the arrow. An interval of 2 min elapsed between the two records during which nicotine was injected and stimulated the superior cervical ganglion. The records show that nicotine prevented the contraction of the nictitating membrane evoked by stimulating the cervical sympathetic nerve but did not modify the respiratory inhibition and vasodepression produced by afferent vagal stimulation. (The maximum frequency of preganglionic cervical sympathetic nerve stimulation capable of producing a sustained response of the nictitating membrane is about 10 Hz. Such frequencies applied to the vagus nerve produce only small vasomotor or respiratory responses, as shown. Hence the vagus nerve was stimulated at two frequencies: 50 Hz to elicit large responses, and 10 Hz to ensure comparability with sympathetic stimulation should any effect of nicotine be frequency dependent.)

Results

The interpretation of the measurements of ganglionic nicotine concentrations described in this paper depend in part on the assumption that nodose ganglion cells are not responsive to nicotine, so that the amounts of nicotine retained in this structure are independent of neuronal response. It seemed necessary, therefore, to confirm Langley & Dickinson's (1889) report of the resistance of the nodose ganglion to nicotine.

Figure 1 shows that close-arterial injection of nicotine blocked transmission through the superior cervical ganglion, indicated by inhibition of the nictitating membrane contraction (middle trace), but did not impair sensory conduction through the nodose ganglion (shown by the persistence of respiratory and vasomotor responses to sensory vagal stimulation). By contrast, ganglion-blocking doses of procaine also reduced these vagal effects. Thus, unless there is a marked difference between the effects of nicotine on axons and somata in the nodose ganglion, these results suggest that nodose ganglion cells are probably unresponsive to nicotine.

^{3}H-nicotine measurements

The concentrations of ^{3}H-nicotine (measured as tritium) in the superior cervical and nodose ganglia were determined 2, 4 and 8 min after injecting 4, 40 or 200 μg of ^{3}H-nicotine close-arterially (Table 1). These concentrations are expressed as % of that in the injection fluid.

With all three doses there was a consistently higher concentration of ^{3}H-nicotine in the superior cervical ganglion than in the nodose ganglion 2 min after injection: the mean overall concentration ratio for all doses, superior cervical ganglion/nodose ganglion (S/N ratio) was 1.60. This ratio declined with increasing times after injection, to 1.21 at 4 min and 0.92 at 8 min.

With the two larger doses of nicotine (40 and 200 μg), ganglion block was complete 2 min after injection, but partial recovery occurred by 8 min. No ganglion block was observed after the 4 μg dose, but stimulation was apparent.

Relation between dose and ganglion ^{3}H-nicotine content

S/N ratios at 2 and 4 min appeared to decline slightly with increasing nicotine doses, but the differences were not significant. The proportions of injected nicotine retained in the ganglia were approximately the same at each dose level. Thus there was no clear evidence of a dose-saturable retention process over this dose range.

^{14}C-inulin measurements

In a further series of experiments a mixture of ^{3}H-nicotine and ^{14}C-carboxyl-inulin was injected and the concentration of each nuclide in the ganglia measured using the simultaneous equation method of Okita, Kabara, Richardson & LeRoy (1957). The object of this was to see to what extent differences in blood supply, penetration rate or fluid compartment spaces might contribute to the differential nicotine concentrations observed in the two ganglia. The results are shown in Table 2.

Measurements of ^{3}H and ^{14}C made 2 min after injection showed two points of dissimilarity between the distribution of nicotine and inulin.

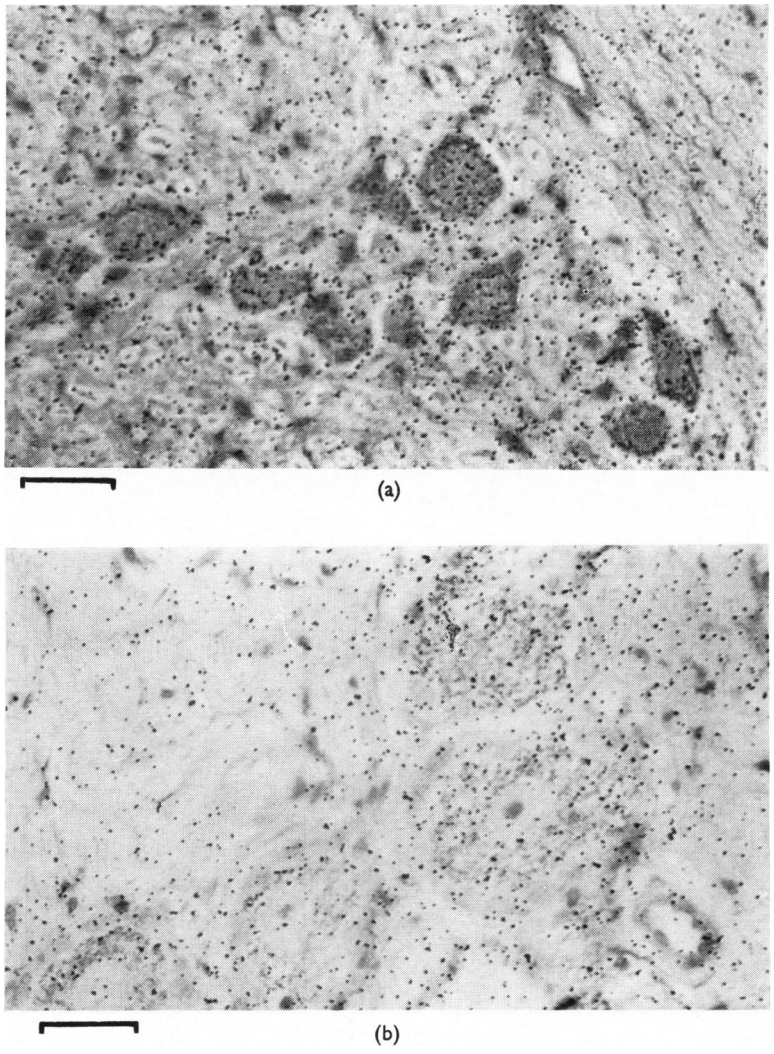

FIG. 2. Autoradiographs of ^{3}H-nicotine in sections of (a) superior cervical and (b) nodose ganglia from the same animal. Frozen, freeze-dried sections (about 0.7 to 0.8 μ thick), unfixed and stained with toluidine blue. Exposure time, 8 weeks. Scale 20 μ. The ganglia were removed 2 min after the close arterial injection of 40 μg of ^{3}H-nicotine. Ganglionic tritium concentrations (measured in part of each ganglion not used for autoradiography) were : superior cervical ganglion, 19,200 d.p.m./mg ; nodose ganglion, 7,600 d.p.m./mg. (The overall grain density depends on several factors, such as the thickness of section and emulsion, conditions of exposure, and conditions and time of photographic development. These conditions were not necessarily identical for the two sections, so overall grain densities do not provide an accurate measure of relative ^{3}H-nicotine contents. It may be noted, however, that the intraneuronal grain density *relative* to the extraneuronal density is greater in the superior cervical ganglion than in the nodose ganglion.)

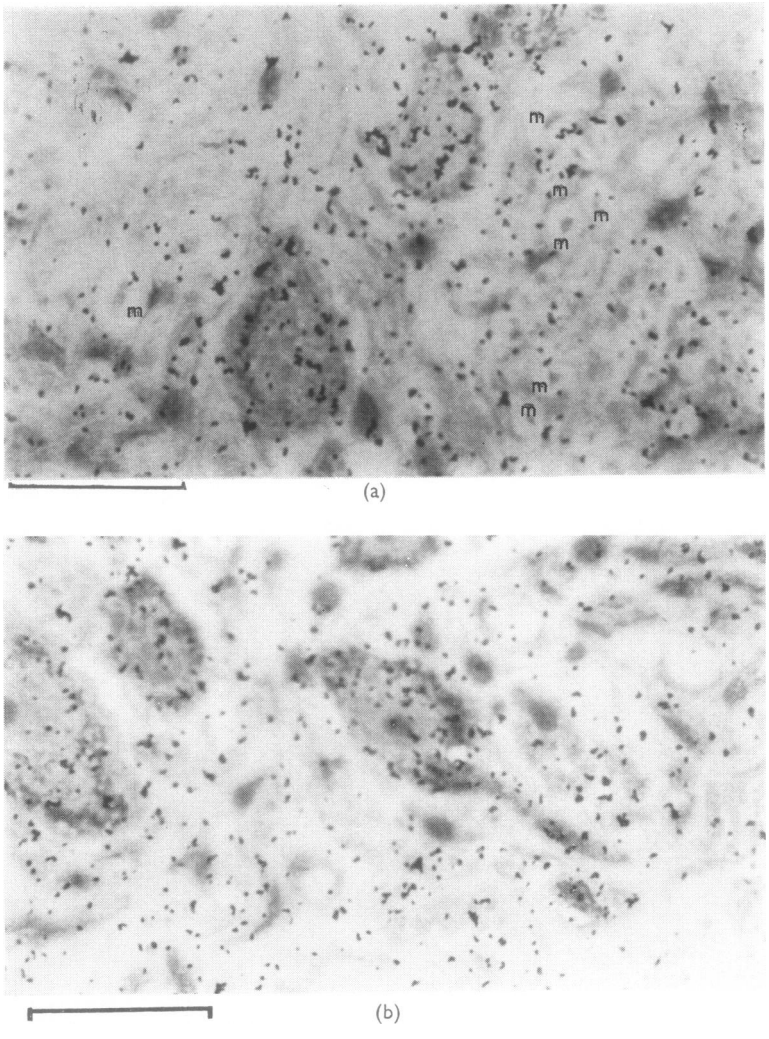

FIG. 3. High power autoradiographs of ^{3}H-nicotine in a cat superior cervical ganglion section (two fields from the same section as that illustrated in Fig. 2(a)). Exposure time, 8 weeks. Scale, 20 μ. Note the high intraneuronal nicotine concentration and the low grain density of single myelinated nerve fibres (m).

TABLE 1. *Concentrations of nicotine retained in cat superior cervical ganglion (S) and nodose ganglion (N) after close-arterial injection of ^{3}H-nicotine*

Dose (μg)	Time (min)	No. of expts.	S response to nicotine: Stimulation* (%)	S response to nicotine: Block† (%)	Nicotine concentration: S	N	S–N	S/N
4 (2·76 μc)	2	5	86·5± 6·5	0± 0	3·50±0·82	2·45±0·80	+1·05±0·44	1·76±0·35
	4	3	97 ± 3	0± 0	1·40±0·76	0·91±0·68	+0·49±0·60	1·37±0·53
40 (26·14 μc)	2	3	64 ± 5	100± 0	4·59±1·23	3·02±0·81	+1·57±0·47	1·52±0·07
	4	3	67 ± 4	100± 0	2·55±0·57	2·25±0·53	+0·30±0·25	1·12±0·13
	8	3	88 ±18	70±18	0·93±0·26	0·99±0·18	−0·06±0·07	0·92±0·07
200 (25·50 μc)	2	3	65 ± 2	100± 0	3·50±1·18	2·85±1·60	+0·65±0·59	1·41±0·24
	4	3	71 ±15	90± 5	2·64±1·80	1·88±0·25	+0·76±0·95	1·14±0·25
	8	2	54, 61	50, 100	0·30, 0·98	0·34, 0·98	−0·006, −0·046	0·99, 0·87
Pooled doses	2	11			3·75±0·49	2·68±0·46	+1·07±0·28	1·60±0·19
	4	9			2·20±0·63	1·68±0·36	+0·52±0·33	1·21±0·19
	8	5			0·82±0·18	0·86±0·16	−0·05±0·07	0·92±0·05

Concentrations are expressed as d.p.m. per mg ganglion/d.p.m. per μl. injection fluid × 100% (mean ±S.E. of mean), and represent the % of the injected concentration present within the ganglia.

* Initial stimulation, % of response of nictitating membrane to preganglionic cervical sympathetic stimulation.

† % reduction of response of nictitating membrane to preganglionic cervical sympathetic stimulation at time of ganglion excision.

First, the concentration of nicotine in the ganglia (expressed as a proportion of the concentration injected) was 10 to 20 times greater than that of inulin. (This is probably the result of an intracellular accumulation of nicotine, see later.)

Second, the S/N ratio for nicotine was greater than 1, as observed in the previous series of experiments, whereas the S/N ratio for inulin was slightly less than 1.

Both these points of difference were less noticeable at later times after injection. Thus, at 8 min, the ganglionic nicotine concentrations were 3 times the inulin concentrations, and the S/N ratios for both substances were unity.

Addition of ^{14}C-carboxyl-inulin to the injection fluid modified somewhat the retention of ^{3}H-nicotine. Thus the concentrations of tritium in the ganglia 4 and 8 min after injection of a mixture of ^{3}H-nicotine with ^{14}C-carboxyl-inulin (Table 2) were substantially lower than those obtained after injecting the same concentration (40 μg) of ^{3}H-nicotine alone (Table 1). Of particular interest is the coincidental reduction of the amount of ganglion block obtained at these times by addition of ^{14}C-carboxyl-inulin (an observation confirmed in other experiments in which the effects of nicotine with and without addition of ^{14}C-carboxyl-inulin were compared in the same cat) and the apparent reduction of the 4 min S/N ratio. The manner in which ^{14}C-carboxyl-inulin affects the retention and pharmacological action of nicotine is being further investigated. Because in this study addition of ^{14}C-carboxyl-inulin did not modify the pharmacological action or retention of nicotine 2 min after injection, the interaction does not affect the interpretation of results obtained at this time after injection.

Since the molecular weight of the ^{14}C-carboxyl-inulin (5000–5500) was greater than that of nicotine (molecular weight, 162), it is possible that the lower ganglionic concentrations of the inulin were due to a slower passage of inulin from blood vessels into tissue fluid. This does not seem to be an important factor, for two reasons. First, autoradiographs of ganglia made 2 min after injecting ^{3}H-inulin showed a widespread distribution of radioactivity throughout the extracellular tissue spaces, with no evidence of confinement to blood vessels (Brown *et al.*, 1969). Second, the ganglionic concentrations of mannitol, a low molecular weight (182) extracellular-space indicator, were similar to those of inulin. 2 min after injecting ^{14}C-(+)-mannitol, the mean ganglionic concentrations of carbon-14 were: sympathetic, $0.41 \pm 0.08\%$ (mean $\pm$ S.E. of mean; $n=4$); nodose, $0.35 \pm 0.01\%$ (compare Table 2). This suggests that the low ganglionic inulin concentration was due to its restricted volume of distribution, rather than to slow penetration. (Mannitol was not routinely used as the extracellular marker substance in experiments where nicotine was administered, for fear that the membrane permeability change induced by nicotine might lead to some intracellular penetration of the mannitol: Creese & Maclagan (1967) have observed a penetration of decamethonium into striated muscle fibres at the end-plate during depolarization by decamethonium. There seemed to be less danger of this with a larger molecule like inulin.)

Water content

The total water content of four pairs of ganglia was determined by drying to constant weight at 85° C and measuring the loss of weight. The mean weight losses of the superior cervical and nodose ganglia were similar (superior cervical ganglion, 79.7%, S.E. of mean, $\pm 0.65\%$ nodose ganglion, 78.9%, S.E., $\pm 1.45\%$).

TABLE 2. *Concentrations of ^{3}H-nicotine and ^{14}C-inulin in ganglia after injecting a mixture of* 40 μg (26·14 μc) *^{3}H-nicotine and* 2·5 *mg* (3·39 μc) *^{14}C-inulin*

Time (min)	No. of expts.	Compound	Sup. cerv. ganglion response: Stimulation %	Sup. cerv. ganglion response: Block %	Nicotine concentration: S	N	S–N	S/N
2	3	^{3}H-nicotine	70±10	100± 0	6·20 ±2·00	4·91 ±2·00	+1·29 ±0·22	1·50±0·25
		^{14}C-inulin			0·383±0·150	0·491±0·218	−0·108±0·022	0·83±0·06
4	3	^{3}H-nicotine	68±18	40±20	0·802±0·217	0·861±0·282	−0·059±0·115	0·96±0·15
		^{14}C-inulin			0·120±0·024	0·113±0·018	−0·006±0·009	0·93±0·09
8	3	^{3}H-nicotine	70± 3	11± 5	0·283±0·076	0·305±0·094	−0·022±0·029	0·98±0·09
		^{14}C-inulin			0·099±0·031	0·102±0·035	−0·003±0·012	1·02±0·13

Concentrations are expressed as d.p.m. per mg ganglion/d.p.m. per μl. injection fluid × 100% (mean ±S.E. of mean).

TABLE 3. *Effect of hexamethonium* (1 *mg close-arterially*) *on the concentration of nicotine retained in the cat superior cervical* (*S*) *and nodose* (*N*) *ganglia* 2 *min after close-arterial injection of* 40 μg *^{3}H-nicotine*

	Control: Nicotine concentration					Plus hexamethonium: Nicotine-concentration			
Expt.	S	N	S–N	S/N	Expt.	S	N	S–N	S/N
1	2·05	1·67	+0·38	1·23	1	4·71	3·53	+1·18	1·34
2	10·77	7·52	+3·25	1·43	2	3·22	2·44	+0·78	1·32
3	2·25	1·48	+0·77	1·52	3	3·94	5·23	−1·29	0·75
4	5·14	4·61	+0·53	1·12	4	2·61	4·39	−1·78	0·59
5	3·34	2·78	+0·56	1·20	5	2·01	2·14	−0·13	0·94
6	1·80	1·27	+0·53	1·42	6	6·62	5·51	+1·11	1·20
7	2·97	2·64	+0·33	1·13	7	2·95	2·95	±0	1·00
Mean	*4·05*	*3·14*	*+0·94*	*1·29*	Mean	*3·72*	*3·74*	*−0·02*	*1·02*
S.E.	*±1·21*	*±0·85*	*±0·39*	*±0·06*	S.E.	*±0·59*	*±0·50*	*±0·44*	*±0·11*

Concentrations are expressed as d.p.m. per mg ganglion/d.p.m. per μl. injection fluid × 100%.

Tritiated water content

The concentrations of ^3H in the ganglia 2 min after close-arterial injection of 10 μc of ^3H_2O (as saline) were measured in three cats. Tritium concentration ratios (S/N ratios) were: 0.85, 0.93 and 0.72 (mean, 0.83).

Effect of hexamethonium

The effect of hexamethonium on ganglionic ^3H-nicotine concentrations measured 2 min after the injection of 40 μg of ^3H-nicotine was determined in seven experiments out of a separate series of fourteen (Table 3). Hexamethonium (1 mg in 0.2 ml.) was injected 1 min before the nicotine injection, control nicotine injections being preceded by 0.2 ml. of saline. This dose of hexamethonium prevented completely ganglion stimulation by the nicotine.

In contrast to the controls, where the ^3H-nicotine S/N ratio was always greater than 1 (as observed previously), the S/N ratio was unity or less in four out of seven cats pretreated with hexamethonium. In the other three hexamethonium-treated animals, however, the S/N ratio was similar to the control ratios, and the mean S/N ratios or (S–N) differences for controls and hexamethonium-treated cats were not significantly different at the 5% level of probability.

Autoradiographic location of ^3H-nicotine

Autoradiographs showed a high intraneuronal concentration of ^3H-nicotine in both superior cervical and nodose ganglia (Figs. 2 and 3). The intraneuronal grain density in the superior cervical ganglion tended to be greater than that in the surrounding tissues (Fig. 2a ; Fig. 3): in the nodose ganglion the grain distribution was more uniform.

These autoradiographs of nicotine contrast strongly with those of inulin, mannitol and Na_2SO_4 prepared by the same technique: the latter show a very low intracellular activity (Brown, Stumpf & Roth, 1969). This reinforces the view that little artefactual translocation resulted during the preparation of the autoradiographs. The distribution of nicotine did resemble that of inulin in one respect, namely that very little nicotine entered myelinated nerve fibres (Fig. 3a).

Discussion

The most interesting observation in these experiments was the presence of a higher concentration of nicotine in the superior cervical ganglion than in the nodose ganglion shortly after injection. The difference was less than that which might have been expected from the autoradiographic picture of Appelgren *et al.* (1963), but was fairly consistent, at a concentration ratio of 1.6.

Because the pharmacological action of nicotine was exerted exclusively on the superior cervical ganglion, the question arises whether the differential distribution of nicotine was associated with this selective action. The evidence so far available suggests that this was so.

First, there was no comparable difference in the amounts of ^{14}C-inulin or ^3H_2O retained in the ganglia. This makes it unlikely that the unequal concentrations of nicotine were the result of different penetration rates to the two ganglia. Also, because the total water contents of the two ganglia were similar, the fairly equal distributions of inulin and injected water suggest that the resting fluid compartment spaces were similar. (The concentrations of inulin in the two ganglia during the peak nicotine block were not quite similar, there being slightly more inulin in the nodose ganglion than in the superior cervical ganglion—that is, in the opposite direction to that seen with nicotine. Whether this has any bearing on the nicotine distribution pattern is not known.)

Secondly, variations in the nicotine distribution pattern followed to some extent the same time course as the pharmacological action. Thus, the highest S/N ratio was observed very shortly after injection when the effect of the nicotine was most pronounced, whereas at 8 min, when the action of even the largest dose of nicotine was waning, the S/N ratio was unity. In this connexion it is of interest that admixture of ^{14}C-inulin to the nicotine both abbreviated the blocking action of the nicotine and concurrently shortened the period during which an elevated S/N ratio was detected.

Thirdly, hexamethonium tended to reduce the S/N ratio. This effect was not consistent, but the inconsistency may have been due to the large variation in the amounts of nicotine retained under the conditions of the experiments: under *in vitro* conditions, where there is much less variance in uptake, hexamethonium consistently reduced the uptake of ^3H-nicotine into rat superior cervical, but not nodose, ganglia (Brown, unpublished observation).

The explanation of this apparent association between the action of nicotine upon the superior cervical ganglion and the amount of nicotine found within the ganglion is not yet clear. Binding to surface receptors can probably be discounted, for with high concentrations of injected nicotine there was no evidence of saturation and the excess concentration of nicotine within the superior cervical ganglion over that found in the nodose ganglion was very great. Thus, with a 200 μg dose of nicotine this excess was up to 15 ng/mg (200 ng/ganglion): if present only on the surface of the neurones such an amount represents a layer of nicotine molecules covering the entire surface at about 10 Å intervals.* In this connexion it may be noted that receptor-binding by agonists on smooth muscle is not readily detectable from uptake measurements (Paton & Rang, 1965). Likewise, while a receptor-dependent uptake of depolarizing agents in striated muscle has been observed, this too cannot be ascribed to receptor-binding (Waser, 1960; Creese, Taylor & Tilton, 1963).

An alternative site of accumulation may be within the neurones. The autoradiographs of ^{14}C-nicotine in the cat superior cervical ganglion published by Appelgren *et al.* (1963) suggested the presence of a high intraneuronal concentration of nicotine. This observation has been confirmed by the higher resolution auto-

* This figure can be derived as follows. The cat superior cervical ganglion contains about 126,000 cells (Billingsley & Ranson, 1918), yielding 10^{-14} g moles of nicotine per cell, or 10^{10} molecules/cell. The mean radius of the neurone soma is about 9 μ (Brown *et al.*, 1969), and surface area therefore $10^3\mu^2$. Assuming the dendrites to increase the surface area to $10^4\mu^2$ (or 10^{12}Å^2), we have 10^{10} molecules/10^{12}Å^2.

radiographs of ^{3}H-nicotine obtained in the present investigation. The presence of substantial amounts of nicotine intracellularly is also indicated by the large difference between the ganglionic nicotine and inulin concentrations.

The autoradiographs show that ^{3}H-nicotine entered the neurones of both sympathetic and nodose ganglia. Relative to the extraneuronal concentrations, however, the intraneuronal nicotine concentration in the superior cervical ganglion appeared to be somewhat higher than that in the nodose ganglion cells. This may also be deduced from the fact that the inulin concentrations in the two ganglia were similar: this suggests that the amounts of extracellular nicotine in the two ganglia were comparable, in which case the extra nicotine in the superior cervical ganglion most likely reflected a greater intraneuronal retention.

A more rapid penetration of nicotine into sympathetic ganglion cells may be expected simply on a basis of relative neuronal surface area, for the superior cervical ganglion cells are smaller than nodose ganglion cells (mean perikaryal diameters, 9 and 14 μ respectively, Brown *et al.*, 1969). This may have some bearing on the present observations, but probably does not fully account for the findings, because a comparable difference in the concentrations of nicotine in sympathetic and nodose ganglia has recently been observed after equilibration of isolated ganglia in nicotine solution (Brown, unpublished observations).

As a tentative hypothesis, it is suggested that there might be an increased intracellular accumulation of nicotine in the superior cervical ganglion consequent on depolarization of the ganglion cells by nicotine. This hypothesis requires substantiation, but is not without precedent, for Creess & Maclagan (1967) found evidence for a local penetration of decamethonium into striated muscle fibres at the end-plate region during decamethonium-induced depolarization. There are some differences between the penetration of decamethonium and nicotine, which depend principally on the fact that the resting cell membrane is essentially impervious to decamethonium but not to nicotine, and it is not yet known whether the two phenomena are truly related.

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