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Intracellular pH in rat isolated superior cervical ganglia in relation to nicotine-depolarization and nicotine-uptake

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Summary

1. The intracellular pH (pH_i) of rat isolated superior cervical ganglia incubated in normal Krebs solution (pH_o=7·37) was estimated to be 7·33 from the uptake of a weak acid, ^{14}C-5,5-dimethyloxazolidine-2,4-dione (DMO). Addition of 30 μM nicotine for 30 min reduced the DMO-estimated pH_i by 0·15 units to 7·18. This effect was prevented by hexamethonium (2·5 mM) or by depolarizing the ganglion with K^+ (124 mM).

2. ^{3}H-Nicotine (30 μM) was concentrated within the ganglia to an intracellular/extracellular concentration ratio (C_i/C_o) of 5·54 in normal Krebs solution and 4·61 in 2·5 mM hexamethonium. This would suggest an intracellular pH of 6·54 and 6·63 respectively. In ganglia previously depolarized by K^+ the corresponding values for C_i/C_o were 4·02 (minus hexamethonium, estimated pH_i 6·95) and 4·17 (plus hexamethonium, estimated pH_i 6·94).

3. A multicompartment cell interior comprising an acid cytoplasm (pH~6·6) and more alkaline nucleus and mitochondria is proposed to explain the difference between the values of pH_i estimated from the uptake of DMO and nicotine. It is suggested that the fall in pH_i during nicotine-depolarization results from metabolic stimulation following Na^+ entry.

Introduction

When isolated rat sympathetic ganglia are soaked in solutions containing nicotine, the nicotine accumulates inside the cells. Intracellular accumulation is increased when nicotine depolarizes the ganglion cells (Brown, Halliwell & Scholfield, 1971).

Nicotine is a weak base, with a pK_a of 8·01 at 25° C (Barlow & Hamilton, 1962). The unionized base appears to penetrate cell membranes quite easily (Weiss, 1966). Consequently the distribution of nicotine across the cell membrane will be greatly affected by the relative pH of the intracellular and extracellular fluids. Thus, intracellular accumulation might be explained if the cell contents were of lower pH (more acid) than that of the extracellular fluid, and depolarization might augment cellular uptake by further reducing intracellular pH.

To check this we have tried to determine the intracellular pH in isolated rat sympathetic ganglia by measuring the distribution of radioactively-labelled 5,5-dimethyloxazolidine-2,4-dione (DMO) between the intra- and extracellular com-

partments. This compound was introduced for the indirect measurement of cell pH by Waddell & Butler (1959): it is a weak acid with a pK_a of 6·33 at 25° C (Addanki, Cahill & Sotos, 1968a).

Methods

The relative concentrations of a weak acid inside (C_i) and outside (C_o) a cell whose membrane is permeable only to the unionized species of the molecule is related to the intracellular pH (pH_i) by the expression

$$pH_i = pK_a + \log\left\{\left[\frac{C_i}{C_o}(1+10^{pH_o-pK_a})\right]-1\right\} \quad \text{(i)}$$

where pH_o is the extracellular pH and pK_a is the negative logarithm of the dissociation constant of the acid (Waddell & Butler, 1959; Irvine, Saunders, Milne & Crawford, 1960). The corresponding equation for a weak base is

$$pH_i = pK_a - \log\left\{\left[\frac{C_i}{C_o}(1+10^{pK_a-pH_o})\right]-1\right\} \quad \text{(ii)}$$

Thus, if the extracellular pH and pK_a are known, the intracellular pH can be calculated from the intracellular/extracellular concentration ratio of the acid or base attained at equilibrium. The latter can be determined by measuring the total tissue uptake and correcting for the extracellular space.

Experimental procedure

Rat superior cervical ganglia were isolated, desheathed, and incubated in 3 to 5 ml of Krebs solution at room temperature (22° to 27° C) bubbled continuously with a mixture of 95% oxygen and 5% carbon dioxide. ^{14}C-5,5-dimethyloxazolidine-2,4-dione and the extracellular space indicator ^{3}H-D-mannitol were added to the bath fluid to give final concentrations of 0·12 mM and 0·04 mM (1 μCi/ml and 20 μCi/ml) respectively. Incubation was continued for a further 30 min, sufficient for equilibration with DMO. The ganglia were then removed and their wet and dry weights and contents of radioactivity determined as described previously (Brown *et al.*, 1971). ^{3}H and ^{14}C were measured to within ±2% by the simultaneous equation method of Okita, Kabara, Richardson & Leroy (1957). During the incubation period the pH of the bath fluid was monitored continuously to within 0·005 units with an E.I.L. Vibret pH meter coupled to a Bryans 27000 potentiometric chart recorder. The addition of DMO to the bath reduced the bath fluid pH by ~0·05 units but this returned to normal in 1 to 2 minutes. This effect presumably resulted from the acidic nature of the compound. Temperature was measured to within 0·1° C with a mercury thermometer. The appropriate pK_a for DMO was obtained from the data of Addanki *et al.* (1968a). Concentrations of DMO were expresed as radioactivity/unit of intracellular and extracellular water and pH_i was calculated from equation (i).

Recovery of added ^{14}C-DMO carried through the extraction procedure was essentially complete: possible variations in recovery were allowed for by subjecting the bath fluid sample to the same extraction procedure as the ganglion before counting. Radiochemical purity of the ^{14}C-DMO and ganglionic metabolism of DMO were checked by subjecting stock ^{14}C-DMO, bath fluid and ganglionic extracts to thin-

layer chromatography using as solvent *n*-butanol: acetic acid: water (25:4:10, v/v). DMO was visualized with 1% mercurous nitrate and radioactivity located by autoradiography and by zonal counting of the silica. Radiochemical purity of the stock ^{14}C-DMO was 98%. Bath fluid and ganglionic radioactivity, extracted in several different solvents, corresponded to at least 80–90% ^{14}C-DMO. The remaining radioactivity was not clearly located at distinct spots, but generally distributed, so may not have represented a specific metabolite. Since the percentage of unchanged DMO in the bath fluid and ganglionic radioactivity did not differ no correction for possible impurities was made.

Tests were made, by methods described previously (Brown *et al.*, 1971), to see whether DMO exerted any pharmacological action on the ganglion. In concentrations up to 0·1 mM DMO did not depolarize the ganglion, nor did it affect the depolarizing action of nicotine.

The effect of nicotine on intracellular pH was tested by adding nicotine to the bath (final concentration 30 μM) 1 min before adding the ^{14}C-DMO. The nicotine was left in contact with the ganglion throughout the 30 min incubation period. Hexamethonium (final concentration 2·5 mM) was added 10 min before the nicotine. When added, nicotine produced a brief rise in bath fluid pH ($<$0·1 units), the pH returning to normal in 1 to 2 minutes. Hexamethonium did not affect bath pH.

Uptake of ^{3}H-nicotine by the ganglion was measured as described previously (Brown *et al.*, 1971).

Solutions. The composition of the Krebs used was (mM): NaCl, 118; KCl, 4·7; $CaCl_2$, 2·52; $NaHCO_3$, 25; KH_2PO_4, 1·18; $MgSO_4$, 1·19; D-glucose, 11. The pH of this solution was usually within the range 7·35–7·45 when bubbled continuously with 5% CO_2. The following modified solutions were used: KCl solution, NaCl replaced with 118 mM KCl, to give (in mequiv./l) 124 [K^+], 25 [Na^+], and 128 [Cl^-]; K_2SO_4 solution, NaCl replaced with 59 mM K_2SO_4, to give (mequiv./l) 124 [K^+], 25 [Na^+], 9·9 [Cl^-] and 59 [SO_4^{2+}]. The pH of these high [K^+] solutions are given in Table 3.

Drugs and radioactive compounds. Radioactive DMO (2-^{14}C-DMO, specific activity, 7·95 mCi/mmole, stated purity$>$98%) was obtained from N.E.N. Gmbh, Germany. ^{3}H-D-mannitol (500 mCi/mmole) and ^{3}H-nicotine (260 mCi/mmole) were obtained from the Radiochemical Centre, Amersham. The proportion of tritium as mannitol and nicotine in stock solutions and in ganglion extracts were determined by thin-layer chromatography as described previously (Brown, Stumpf & Roth, 1969; Brown, Hoffmann & Roth, 1969). Purities were in excess of 98% and 90% respectively. Unlabelled DMO and D-mannitol were obtained from Eastman and Koch-light respectively.

Nicotine and hexamethonium were added to the bath as free base and bromide salt respectively.

Assumptions and errors. Several assumptions concerning the distribution of DMO are involved in estimating intracellular pH by this method (Waddell & Butler, 1959; Butler, Waddell & Poole, 1967), principally that (1) only the unionized molecules cross the cell membranes, with no material leakage of ionized

molecules; (2) the distribution of the unionized DMO across the cell membrane attains equilibrium during the incubation period; (3) DMO is not actively transported or bound by tissue components; (4) it is not metabolized; (5) it is pharmacologically inert; and (6) the concentration used is insufficient to affect cell pH. These assumptions are valid in other tissues (Butler *et al.*, 1967; Addanki *et al.*, 1968a). Assumptions 2, 4, 5 and 6 were also checked and found to hold for isolated ganglia.

The principal source of experimental error is probably the measurement of the extracellular space (Addanki, Cahill & Sotos, 1968b). The reason is that the intracellular concentration of DMO is usually lower than the extracellular concentration so the same difficulty is presented as (for example) in measuring accurately intracellular Na^+ concentrations. A further problem is that the apparent extracellular space occupied by different markers in ganglia varies with the molecular weight of the marker (Brown *et al.*, 1971). For this reason mannitol was used in preference to inulin. Even so, since the molecular weight of mannitol (180) is greater than that of DMO (127), the extracellular space available to DMO might be underestimated. An underestimate of 5% in the DMO-extracellular space would lead to an overestimate of the intracellular pH of about 0·02 unit. Within the pH_i range observed (7·1 to 7·3), an error in pK_a for DMO of 0·1 unit would affect the calculated value for pH_i by less than 0·01 unit, and so can be considered an insignificant source of error.

Results

Intracellular pH in normal Krebs solution

After incubating isolated rat superior cervical ganglia for 30 min in normal Krebs solution containing ^{14}C-DMO the concentration ratio between the intra- and extracellular fluids (C_i/C_o) was $0{\cdot}93 \pm 0{\cdot}04$ (mean ± S.E. of mean of 10 determinations, Table 1). The mean intracellular pH (pH_i) calculated from this distribution ratio according to equation (i), was $7{\cdot}33 \pm 0{\cdot}02$ at a mean extracellular pH (pH_o) of $7{\cdot}37 \pm 0{\cdot}02$. The mean pH gradient ($pH_o - pH_i$) across the cell membrane ($0{\cdot}04 \pm 0{\cdot}02$) was not significantly different from zero ($P = 0{\cdot}1 > 0{\cdot}05$ by Student's t test).

Nicotine made the cell interior more acid. In the presence of 30 μM nicotine, C_i/C_o for DMO was reduced by $26{\cdot}1 \pm 1{\cdot}5\%$ and pH_i by $0{\cdot}15 \pm 0{\cdot}01$ units, to a mean value of $7{\cdot}18 \pm 0{\cdot}02$ (Table 1). Since pH_o was not significantly affected by the nicotine (apart from the brief fluctuation on adding the nicotine noted in **Methods**) the reduced pH_i was associated with a corresponding increase in the pH gradient across the cell membrane.

This effect of nicotine on cell pH was associated with an action on acetylcholine-receptors, for two reasons:

(*a*) Hexamethonium, in a concentration (2·5 mM) which annuls nicotine-depolarization, prevented the effect of nicotine on pH_i (Table 1). Hexamethonium alone did not affect pH_i.

(*b*) Nicotine does not depolarize cells in the nodose (afferent vagal) ganglion, nor is nicotine uptake in this ganglion sensitive to hexamethonium (Brown *et al.*, 1971).

TABLE 1. *Effect of nicotine* (30 μM) *on* ^{14}*C-DMO* (C_i/C_o) *uptake and calculated intracellular pH* (pH_i) *in rat isolated superior cervical ganglia incubated in normal Krebs solution and in Krebs solution containing* 2·5 mM *hexamethonium* (*means*±S.E., n=*number of determinations*)

	Solution	*n*	C_i/C_o	pH_o	pH_i	pH_o–pH_i
Normal Krebs	(a) Control	10	0·933±0·043	7·370±0·015	7·334±0·018	+0·039±0·019
	(b) Nicotine	10	0·687±0·028	7·362±0·014	7·178±0·015	+0·194±0·020
	*b/a	10	0·739±0·015†			
	*b–a	10		—0·008±0·008	—0·153±0·014†	+0·159±0·011
Hexamethonium	(a) Control	6	0·945±0·075	7·327±0·012	7·289±0·037	+0·038±0·040
	(b) Nicotine	6	0·985±0·069	7·323±0·013	7·290±0·025	+0·023±0·035
	*b/a	6	1·013±0·012			
	*b/a	6		—0·004±0·003	+0·002±0·027	—0·015±0·013

* In paired ganglia from the same rat. † Significant change ($P<0·001$).

Likewise, in isolated rat nodose ganglia nicotine did not reduce the cellular uptake of ^{14}C-DMO or intracellular pH calculated from the uptake (Table 2).

In nodose ganglia and also in sympathetic ganglia treated with hexamethonium, nicotine tended to increase cell pH slightly. This is probably because relatively high concentrations of nicotine accumulate within the cells (>100 μM, see below) and exert a direct effect on pH as a base, in the manner reported for skeletal muscle (Weiss, 1968). If this effect is allowed for, then the fall in pH_i associated with the depolarizing action of nicotine would be slightly greater than that shown in Table 1.

Intracellular pH in K^+ solution

When isolated ganglia are soaked for 30 min in 30 μM nicotine solution, the intracellular Na^+ concentration is increased by some 50 mM, with a corresponding decrease of intracellular [K^+] (C. N. Scholfield, unpublished observations). Since intracellular pH may be related to the intracellular concentrations of Na^+ and K^+ (Waddell & Bates, 1969), these ionic changes may play some part in the reduction of pH_i during nicotine depolarization. To test this, the effect of nicotine on cell pH was measured in ganglia previously depolarized by K^+ ions, in which little change of Na^+ and K^+ during application of nicotine would occur. Also, the effect of K^+-depolarization itself on cell pH might indicate how far the fall in pH_i produced by nicotine resulted simply from the change in membrane potential.

In these experiments the K^+ concentration of the bath fluid was raised to 124 mM by replacing NaCl in the Krebs solution with either KCl or K_2SO_4. The ganglia were soaked in these solutions for 2 to 3 hours before adding ^{14}C-DMO, to obtain full equilibration of intracellular and extracellular Na^+ and K^+. As might be expected from the observations of Boyle & Conway (1941) on frog muscle, substitution of KCl for NaCl made the ganglion cells swell, their water content being approximately doubled. In K_2SO_4 solution the cells swelled by about 12% of their volume in normal Krebs solution.

The intracellular DMO concentration was reduced by about half in these K^+ solutions, indicating a much greater pH gradient between the extra- and intracellular environments than in normal Krebs solution (Table 3, compare with Table 1). In KCl solution this resulted from a fall of pH_i since pH_o was similar to that in normal Krebs solution. In K_2SO_4 solution pH_i was not reduced, the increased pH gradient resulting from the high pH of the bathing medium.

TABLE 2. *^{14}C-DMO uptake and calculated intracellular pH in rat isolated nodose (afferent vagal) ganglia incubated in normal Krebs solution (means ±S.E., n=number of estimates). (Nodose ganglia were incubated together with the sympathetic ganglia referred to in Table 1: pH_o and nicotine concentration are therefore those in Table 1)*

Solution	*n*	C_i/C_o	pH_i	pH_o–pH_i
(a) Control	9	0·792±0·032	7·249±0·018	+0·114±0·019
(b) Nicotine	10	0·817±0·040	7·256±0·019	+0·104±0·022
* b/a	9	1·049±0·032		
* b—a	9		+0·008±0·015	—0·018±0·015

* In paired ganglia.

TABLE 3. *Effect of nicotine* (30 μM) *on* ^{14}C*-DMO uptake and calculated intracellular pH in rat isolated superior cervical ganglia incubated in* K^+ *solution* (*means* ± S.E., n = *number of determinations*)

	Solution	*n*	C_i/C_o	pH_o	pH_i	$pH_o - pH_i$
KCl	(a) Control	4	0·401 ± 0·039	7·435 ± 0·156	6·943 ± 0·045	+0·493 ± 0·065
	(b) Nicotine	4	0·394 ± 0·044	7·425 ± 0·136	6·933 ± 0·050	+0·498 ± 0·067
	*b/a	4	0·979 ± 0·029			
	*b–a	4		−0·010 ± 0·010	−0·010 ± 0·012	+0·005 ± 0·012
K_2SO_4	(a) Control	8	0·505 ± 0·015	7·686 ± 0·024	7·368 ± 0·019	+0·323 ± 0·013
	(b) Nicotine	8	0·543 ± 0·018	7·651 ± 0·020	7·365 ± 0·018	+0·290 ± 0·016
	*b/a	8	1·080 ± 0·041			
	*b–a	8		−0·035 ± 0·028	−0·003 ± 0·034	−0·040 ± 0·020

* Paired ganglia.

Nicotine did not reduce DMO uptake or intracellular pH calculated from the uptake in K^+ solution, either when pH_i was already reduced (in KCl solution) or when it was not (in K_2SO_4 solution).

Uptake of ^{3}H-nicotine

Since depolarization by K^+ ions prevented the effect of nicotine on the DMO-calculated pH_i it might also prevent the increased nicotine uptake associated with nicotine depolarization seen in normal Krebs solution (Brown *et al.*, 1971). To test this, isolated ganglia were incubated for 30 min in either normal Krebs solution or K_2SO_4 solution containing ^{3}H-nicotine (30 μM) and their content of ^{3}H-nicotine measured. To distinguish the effect of nicotine depolarization (or nicotine-receptor interaction) on nicotine uptake, accumulation of nicotine in the presence and absence of 2·5 mM hexamethonium was compared in paired ganglia from the same rat.

In normal Krebs solution mean C_i/C_o for nicotine in the presence of hexamethonium (i.e., in the absence of depolarization) was 4·61 ± 0·14 (Table 4). Without hexamethonium (i.e., with depolarization) this increased to 5·54 ± 0·19. Both values were somewhat lower than those found previously (Brown *et al.*, 1971) but the fraction by which uptake was increased during depolarization (20·6 ± 4·0%) was similar in the two series of experiments.

In ganglia depolarized by K_2SO_4, nicotine uptake in the presence of hexamethonium was not significantly different from that in normal Krebs solution with hexamethonium. However, depolarization by K_2SO_4 prevented the increase of nicotine uptake previously observed on omitting hexamethonium.

The mannitol space was not measured in these experiments so individual values for pH_i based on nicotine distribution could not be calculated. Mean values for the calculated pH_i, estimated from the values for pH_o and mannitol space obtained in previous experiments, were considerably lower than those suggested by the distribution of DMO under all conditions tested (Table 4).

TABLE 4. *Uptake of ^{3}H-nicotine and calculated pH_i and pH gradient in rat isolated superior cervical ganglia incubated in normal Krebs solution. Ganglia were incubated for 30 min in 30 μM ^{3}H-nicotine (a) in the absence and (b) in the presence of 2·5 mM hexamethonium to reveal the effect of depolarization*

	Solution	*n*	C_i/C_o mean ± S.E.	‡pH_i mean	‡pH_o–pH_i
Normal Krebs	(a) —hexamethonium	8	5·54 ± 0·19	6·54	0·73
	(b) +hexamethonium	8	4·61 ± 0·14	6·63	0·82
	† a/b	8	*1·206 ± 0·040		
	† a–b	8		—0·09	+0·09
K_2SO_4	(a) —hexamethonium	8	4·02 ± 0·20	6·95	0·74
	(b) +hexamethonium	8	4·17 ± 0·18	6·94	0·75
	†a/b	8	0·968 ± 0·035		
	†a–b	8		—0·01	+0·01

*Significant change ($P < 0·001$). †Between paired ganglia.
‡Based on average pH_o of 7·37 in normal Krebs solution and 7·69 in K_2SO_4 solution.

Discussion

Intracellular pH and nicotine uptake at rest

In non-depolarized ganglia the intracellular pH calculated from the distribution of DMO was 7·33 at an extracellular pH of 7·37 (Table 1). The intracellular: extracellular concentration ratio (C_i/C_o) for nicotine appropriate to this pH difference should be 1·08. However, the observed concentration ratio for ^{3}H-nicotine (measured when depolarization was blocked by hexamethonium) was 4·61 (Table 4): if due to the pH gradient this would require an intracellular pH of about 6·6.

A similar discrepancy between the intracellular concentrations of nicotine and DMO has been reported in isolated salivary glands (Putney & Borzelleca, 1971). These investigators suggested that the high nicotine concentration ratio resulted from some factor other than the pH gradient, for example, selective dissolution in non-aqueous components (reflecting its lipid solubility) or selective intracellular binding. Neither seems very likely in ganglia, because (*a*) autoradiographs have shown that nicotine tends to be excluded from, rather than concentrated in, such lipid elements as myelin (Schmiterlöw, Hansson, Andersson, Appelgren & Hoffmann, 1967; Brown *et al.*, 1969a) and (*b*) the uptake of nicotine by ganglion cells is proportional to the external concentration over a wide concentration range, which argues against a finite number of binding sites (Brown *et al.*, 1971).

An alternative interpretation is that different regions of the cell have different H^+ ion concentrations (cf., Waddell & Bates, 1969). This seems quite probable from measurements in other tissues. The pH of neural cytoplasm appears to be rather low, between 0·5 and 0·9 units below that of the external medium when measured directly with H^+-sensitive microelectrodes (Caldwell, 1958; Spyropoulos, 1960; Sorokina, 1965). Indicator dyes injected into living *Arbacia* eggs or living *Amoeba* suggest a comparable cytoplasmic pH (6·6–6·8), but a much higher nuclear pH (7·6–7·8; Chambers & Chambers, 1962). Isolated mitochondria may be even more alkaline, up to pH 8·6 (Addanki *et al.*, 1968a).

Regional differences of this magnitude could well account for the different pH values obtained from the uptake of nicotine and DMO. In such a heterogeneous system the relative concentrations of nicotine or DMO in each compartment can be calculated from the appropriate form of the Henderson–Hasselbalch equation, rearranged to read

$$\text{for a base: } \log([BH^+]/[B]) = pK_a - pH$$

$$\text{and, for an acid: } \log([A^-]/[AH]) = pH - pK_a$$

At equilibrium, the concentrations of unionized base (B) or acid (AH) may be assumed equal in all cell compartments and in the extracellular fluid. The total intracellular concentrations of nicotine or DMO will also depend on the volumes of each compartment. Electron micrographs of isolated rat ganglia (D. L. Tamarind, unpublished pictures) suggest the following approximate volumes, as a percentage of the total cell volume: cytoplasm 88%; nucleus, 10%; mitochondria, 2%. Assuming pH values similar to those above, e.g., cytoplasm 6·6, nucleus 7·7 and mitochondria 8·6, then at an extracellular pH of 7·37 the final concentration ratio C_i/C_o for nicotine would be 4·61 and the apparent intracellular pH 6·65. On the other hand, the final concentration ratio for DMO would be 0·68, giving an apparent intracellular pH of 7·18.

Thus, the high intracellular concentration of nicotine may result from a low cytoplasmic pH of about 6·6, whereas the uptake of DMO may be more dependent upon the volume and pH of more alkaline cell inclusions such as nucleus and mitochondria. Experiments to check this with other acids and bases are in progress: preliminary results with morphine accord with the pH value predicted from nicotine uptake. Some additional support for this 'multicompartment' hypothesis is provided by previous autoradiographs of labelled nicotine in ganglion cells (Appelgren, Hansson & Schmiterlöw, 1963; Brown *et al.*, 1969a), which show a higher grain density in the peripheral cytoplasm than in the nucleus.

Effect of nicotine-depolarization on intracellular pH

Nicotine-depolarization reduced the uptake of DMO and increased the uptake of ^{3}H-nicotine. Both could be explained by a reduced intracellular pH of 0·15 and 0·09 units respectively. However, the relationship between these two effects is obscured by the difference in the resting pH values indicated by the uptakes of DMO and nicotine. If our multicompartment explanation for this discrepancy is correct, the increased nicotine uptake could most easily be explained by a fall in cytoplasmic pH of about 0·1 units whereas the reduced uptake of DMO might arise from simultaneous acidification of extracytoplasmic components.

It seems unlikely that nicotine reduced the pH solely through membrane depolarization, since nicotine uptake was not increased by depolarizing the ganglion with K^+ ions. Instead the effect of nicotine on cell pH might be mediated through the influx of Na^+ ions, which would increase the metabolic rate of the cells (McIlwain, 1966). In ganglia analogous metabolic stimulation following trains of action potentials consists principally of an increased rate of glycolysis, with a consequent accumulation of lactic acid (Dolivo & Larrabee, 1958). Although long exposures to nicotine were necessary to ensure equilibration of nicotine and DMO between the intra- and extracellular fluids, hexamethonium also reduces the ganglionic uptake of ^{3}H-nicotine measured after only 5 min exposure to nicotine *in vitro* (Brown & Halliwell, unpublished observations) or after injecting nicotine close-arterially *in vivo* (Brown *et al.*, 1969a). Thus, nicotine depolarization may have quite a rapid effect on cell pH.

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REFERENCES

ADDANKI, S., CAHILL, F. D. & SOTOS, J. F. (1968a). Determination of intramitochondrial pH and intramitochondrial—extramitochondrial pH gradient of isolated heart mitochondria by the use of 5,5-dimethyl-2,4-oxazolidinedione. *J. biol. Chem.*, **243**, 2337–2348.

ADDANKI, S., CAHILL, F. D. & SOTOS, J. F. (1968b). Reliability of the quantition of intramitochondrial pH and pH gradient of heart mitochondria. *Anal. Biochem.*, **25**, 17–29.

APPELGREN, L.-E., HANSSON, E. & SCHMITERLÖW, C. G. (1963). Localization of radioactivity in the superior cervical ganglion of cats following injection of ^{14}C-labelled nicotine. *Acta physiol., scand.*, **59**, 330–336.

BARLOW, R. B. & HAMILTON, J. T. (1962). Effect of pH on the activity of nicotine and nicotine monomethiodide on the rat diaphragm preparation. *Br. J. Pharmac. Chemother.*, **18**, 543–549.

BOYLE, P. J. & CONWAY, E. J. (1941). Potassium accumulation in muscle and associated changes. *J. Physiol., Lond.*, **100**, 1–63.

BROWN, D. A., HALLIWELL, J. V. & SCHOLFIELD, C. N. (1971). Uptake of nicotine and extracellular space indicators by isolated rat ganglia in relation to receptor activation. *Br. J. Pharmac.*, **42**, 100–113.

BROWN, D. A., HOFFMANN, P. C. & ROTH, L. J. (1969). ^{3}H-Nicotine in cat superior cervical and nodose ganglia after close-arterial injection *in vivo*. *Br. J. Pharmac.*, **35**, 406–417.

BROWN, D. A., STUMPF, W. E. & ROTH, L. J. (1969). Locatien of radioactivity-labelled extracellular fluid indicators in nervous tissue by autoradiography. *J. cell. Sci.*, **4**, 265–288.

BUTLER, T. C., WADDELL, W. J. & POOLE, D. T. (1967). Intracellular pH based on the distribution of weak electrolytes. *Fedn Proc.*, **26**, 1327–1332.

CALDWELL, P. C. (1958). Studies on the internal pH of large muscle and nerve fibres. *J. Physiol., Lond.*, **142**, 22–62.

CHAMBERS, R. & CHAMBERS, E. L. (1962). *Explorations into the Nature of the Living Cell.* Cambridge, Mass: Harvard University Press.

DOLIVO, M. & LARRABEE, M. G. (1958). Metabolism of glucose and oxygen in a mammalian sympathetic ganglion at reduced temperature and varied pH. *J. Neurochem.*, **3**, 72–88.

IRVINE, R. O. H., SAUNDERS, S. J., MILNE, M. D. & CRAWFORD, M. A. (1960). Gradients of potassium and hydrogen ion in potassium deficient voluntary muscle. *Clin. Sci.*, **20**, 1–18.

MCILWAIN, H. (1966). *Biochemistry and the Central Nervous System.* 3rd Ed. London: Churchill.

OKITA, G. T., KABARA, J. J., RICHARDSON, F. & LEROY, G. V. (1957). Assaying compounds containing ^{3}H and ^{14}C. *Nucleonics*, **15**, 11–114.

PUTNEY, J. W., Jr. & BORZELLECA, J. F. (1971). On the mechanisms of ^{14}C-nicotine distribution in rat submaxillary gland *in vitro*. *J. Pharmac. exp. Ther.*, **178**, 180–191.

SCHMITERLÖW, C. G., HANSSON, E., ANDERSSON, G., APPELGREN, L.-E. & HOFFMANN, P. C. (1967). Distribution of nicotine in the central nervous system. *Ann. N.Y. Acad. Sci.*, **142**, 2–14.

SPYROPOULOS, C. S. (1960). Cytoplasmic pH of nerve fibres. *J. Neurochem.*, **5**, 185–194.

SOROKINA, A. A. (1965). Izmerenie aktivnosti ionov voderada vne i vnetri nervnykh kletok gangliev mollyuskov. *Zh. Evolyyutsionoi Biokhim. i Fisiol.*, **1**, 342–350. (Cited by Waddell, W. J. & Bates, R. G. (1969). *Physiol. Rev.*, **49**, 285–329.).

WADDELL, W. J. & BATES, R. G. (1969). Intracellular pH. *Physiol. Rev.*, **49**, 285–329.

WADDELL, W. J. & BUTLER, J. V. (1959). Calculation of intracellular pH from the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO). Application to skeletal muscle of the dog. *J. clin. Invest.*, **38**, 720–729.

WEISS, G. B. (1966). The effect of pH on nicotine-induced contracture and Ca^{45} movements in sartorius muscle. *J. Pharmac. exp. Ther.*, **154**, 605–612.

WEISS, G. B. (1968). Dependence of nicotine-C^{14} distribution and movements upon pH in frog sartorius muscle. *J. Pharmac. exp. Ther.*, **160**, 135–147.

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