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Effects of norepinephrine, acetylcholine and calcium on the oxidation of glucose in the submaxillary gland of the rat

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IN A prior communication[1] which pertained primarily to the oxidation of glucose labeled in C-1, it was stated that norepinephrine or acetylcholine with eserine *in vitro* stimulated the production of $^{14}CO_2$ from radioactive glucose by submaxillary gland slices of rat, but only in the presence of calcium. The present report extends the comparison of the oxidation to carbon dioxide of glucose labeled in C-1 and C-6, under conditions in which some of the pathways of normal metabolism were blocked. Submaxillary (submandibular) glands from mature male Sprague-Dawley rats were used, and the procedures for tissue preparation, incubation and measurement of $^{14}CO_2$ production were the same as described previously.[1]

RESULTS

The results of eight separate experiments are shown in Fig. 1. Each grouping shows the effects of either norepinephrine (295 μM) or acetylcholine (342 μM, with 360 μM eserine), of iodoacetic acid alone (10 mM), and of the combination of iodoacetic acid with norepinephrine or acetylcholine (plus eserine). Each control value (when nothing extra was added) is presented as 100%, so each response greater than that represents a stimulation, whereas anything below 100% shows an inhibitory effect.

It may be noted, as previously reported,[1] that when calcium was omitted from the incubation medium, neither norepinephrine nor acetylcholine had any effect on $^{14}CO_2$ production when glucose-1-^{14}C (G-1-^{14}C) was the substrate (Fig. 1-A). With glucose-6-^{14}C (G-6-^{14}C) as the substrate under the same conditions, norepinephrine and acetylcholine both had inhibitory effects of approximately 20% (Fig. 1-B).

With calcium included in the incubation medium in the normal amount for Krebs-Ringer bicarbonate (2·54 mM), the stimulatory effect of norepinephrine was considerable, whereas that of acetylcholine was less, but still definite, both with G-1-^{14}C as the substrate (Fig. 1-C and Ref. 1) and with G-6-^{14}C (Fig. 1-D). Norepinephrine did not always have as great a stimulation on the $^{14}CO_2$ production as in these particular experiments (90% or more), but its effect was usually greater than that of acetylcholine plus eserine.

Iodoacetic acid alone at a concentration of 10 mM caused considerable inhibition of $^{14}CO_2$ production with either substrate, and with or without added calcium (Fig. 1). The inhibition was greater with G-6-^{14}C (88–95%) than with G-1-^{14}C (67–86%).

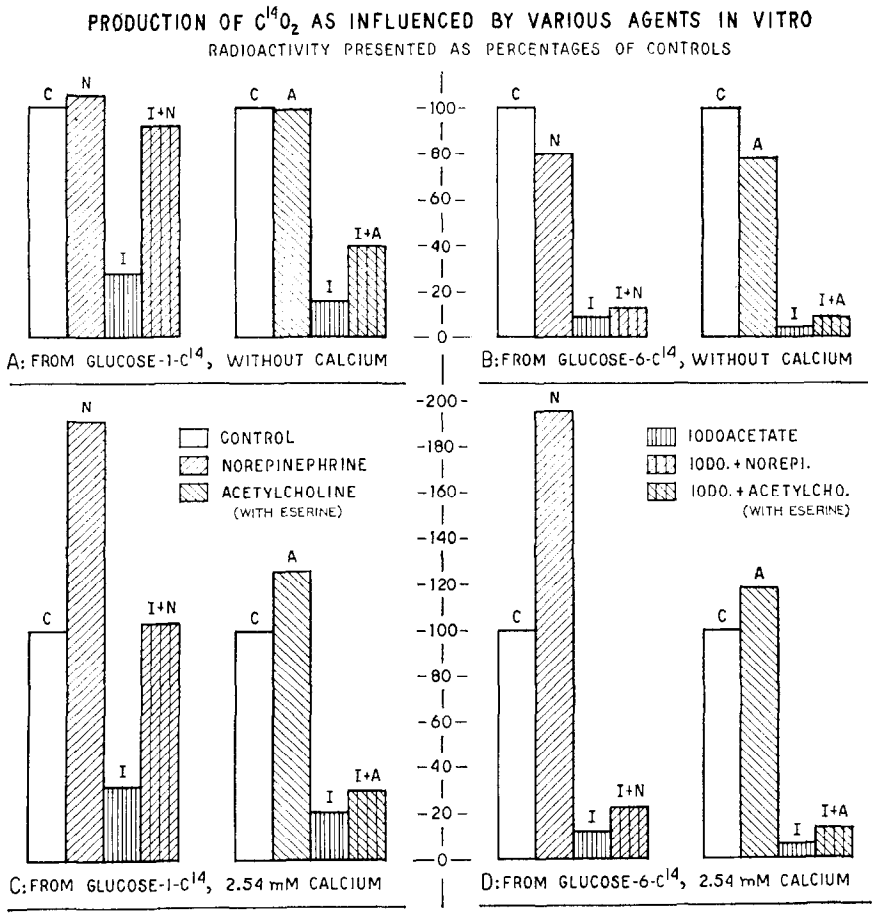

FIG. 1. Slices of male rat submaxillary glands were incubated in Krebs-Ringer bicarbonate buffer at pH 7·4. Each flask contained 1 mg D-glucose as the substrate, with 0·25 μc radioactivity in glucose-^{14}C, at C-1 or C-6, as indicated. The test agents were used in the incubation medium in the following concentrations: norepinephrine (N), 295 μM; acetylcholine (A), 342 μM (with 360 μM eserine included); and iodoacetic acid (I), 10 mM. Calcium was included at 2·54 mM, or omitted, as indicated. The shading code for the graph bars applies to all eight experiments.

The counts per minute (cpm) were recorded after trapping the $^{14}CO_2$ that was formed from glucose oxidation in 45-min incubation at 37°. Each control count (calculated per 100 mg tissue) is presented as a 100% response. The average cpm/100 mg obtained with each agent or combination of agents was converted to a percentage of the respective control cpm, for easier comparison from one experiment to another. (The control cpm/100 mg tissue for the eight experiments ranged from 2,154 to 4,079.) Each bar represents the average value for two determinations, and each group of four bars constitutes a single experiment.

When G-1-^{14}C was the substrate and norepinephrine or acetylcholine was included simultaneously with iodoacetic acid, the production of $^{14}CO_2$ from radioactive glucose was more than when the inhibitor was used alone; i.e. there was "stimulation" by the neurohumors (Fig. 1-A, 1-C). With G-6-^{14}C as the substrate, however, the norepinephrine and acetylcholine had only minor effects in the presence of the iodoacetate (Fig. 1-B, 1-D). Norepinephrine undoubtedly has a much greater stimulatory effect on the oxidation of G-1-^{14}C than of G-6-^{14}C, both without and with calcium, in the

presence of the inhibitor. (Compare Fig. 1-A with 1-B, and 1-C with 1-D.) This stimulation by norepinephrine was independent of the addition of calcium to the medium (Fig. 1-A, 1-C). Acetylcholine in the presence of the inhibitor appeared also to stimulate G-1-^{14}C oxidation more than G-6-^{14}C oxidation, especially without calcium present (Fig. 1-A, 1-B), but the differences were not so striking as with norepinephrine.

DISCUSSION

The yield of $^{14}CO_2$ from the metabolism of G-6-^{14}C can be interpreted to indicate oxidation primarily through the Embden-Meyerhof-Krebs (E-M-K) pathway, and that from G-1-^{14}C to represent activity of the E-M-K or the pentose monophosphate pathway or of both.[2] Although there are limitations to the derivation of quantitative conclusions from these data,[3] it seems that the almost equal yield of radioactivity in $^{14}CO_2$ from equivalent amounts of G-1-^{14}C and G-6-^{14}C, as we reported,[1] would indicate that, *in vitro*, the E-M-K pathway is predominant in the rat submaxillary gland. Such an interpretation is in accord with that of Goldman and co-workers, who have likewise recently reported on a study of glucose metabolism in the rat submaxillary gland.[4]

When submaxillary gland tissue was incubated under normal conditions with no inhibitor, the presence of calcium appeared to be required for norepinephrine or acetylcholine (with eserine) to exert a stimulatory effect on glucose oxidation to CO_2, presumably through the E-M-K pathway. Since norepinephrine or acetylcholine apparently caused a partial inhibition (about 20%) of $^{14}CO_2$ production from G-6-^{14}C, but not from G-1-^{14}C, when calcium was omitted from the buffer medium, this finding could be indicative of a slight shift in glucose utilization from the E-M-K pathway to the pentose monophosphate pathway, without having affected the total oxidation.

The most interesting finding that arose from the use of the iodoacetic acid was that norepinephrine (and also acetylcholine with eserine to a lesser extent) was able to counter or overcome a large portion of the inhibition with G-1-^{14}C as the substrate, but not with G-6-^{14}C. Iodoacetate is believed to inhibit glycolysis primarily, and especially phosphoglyceraldehyde dehydrogenase, but the inhibition is certainly not specific[5] at the relatively high concentration of 10 mM. Therefore it seems likely that the "stimulation" of radioactive carbon dioxide production by norepinephrine found in the presence of iodoacetate is the result of an increase in the activity of the pentose monophosphate pathway. Furthermore, this increased activity brought about by norepinephrine, and possibly also by acetylcholine, showed no evidence of being calcium dependent.

There is an indication from a small number of unpublished data with acetate-1-^{14}C as the substrate that the presence or absence of calcium apparently has no effect on whatever slight and uncertain stimulation norepinephrine, or acetylcholine with eserine, might have on the production of $^{14}CO_2$. This observation is consistent with the idea suggested by the above findings that calcium is required for the action of norepinephrine or acetylcholine in stimulating Embden-Meyerhof glycolysis, prior to the entry of glucose metabolites into the Krebs citric acid cycle.

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